.9
69P
no.2

Library

THE PLANT DISEASE REPORTER

Issued By

LIBRARY
CURRENT SERIAL RECORD
★ OCT 29 1956 ★
DEPARTMENT OF AGRICULTURE

PLANT DISEASE EPIDEMICS
and
IDENTIFICATION SECTION

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

Volume 40 Number 10

October 15, 1956

The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Plant Disease Epidemics and Identification Section serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

THE PLANT DISEASE REPORTER

Issued by

PLANT DISEASE EPIDEMICS AND IDENTIFICATION SECTION

Horticultural Crops Research Branch — Plant Industry Station, Beltsville, Maryland
Volume 40 — October 15, 1956 — Number 10

IN THIS ISSUE

HOWARD R. GARRISS and J. C. WELLS report the first known occurrence in the Western Hemisphere of the parasitic flowering plant Striga sp., associated with poor growth and dying of corn in North Carolina, page 837.

Soil Fumigation: J. G. BALD and R. N. JEFFERSON have formulated a mathematical model to aid in interpretation of results from soil fumigation, page 840. BERT LEAR reports investigations on diffusion in the soil of Vapam applied by various means, primarily for nematode control, page 847. Root growth was better and ears were larger in plots of corn treated for control of parasitic nematodes, according to J. Y. OAKES et al., page 853. WILLIAM LAUTZ summarizes a 5-year study of soil treatment for tobacco black shank control, page 855.

Nematodes (see also under soil fumigation): HARRY W. FORD reports that of 133 chemicals screened for possible systemic action against the citrus disease spreading decline, none has so far shown promise, page 861. Studies on the host range of the burrowing nematode are reported by WRAY BIRCHFIELD, page 866. W. R. JENKINS et al. report occurrence of cyst nematodes in Maryland, page 869.

Fruit Disease Control: Certain contact fungicides effectively prevented sporulation of the scab fungus in holdover stromata on dormant pecan nursery trees, according to RICHARD H. CONVERSE, page 870. J. R. KIENHOLZ reports that study of weather relations and latent infection has led to more effective control of bull's-eye rot of pome fruits in the Pacific Northwest, page 872.

Cereal Diseases (see also GARRISS and WELLS, OAKES et al., McKINNEY): LAURENCE H. PURDY summarizes data indicating that effectiveness of wheat seed treatment for smut control is not improved by storage of treated seed, page 878. Observations on the epidemiology of wheat rusts in Kansas in 1955 are reported by S. M. PADY and C. O. JOHNSTON, page 882. FRANCIS J. LeBEAU et al. obtained a basis for estimating the amount of loss from wheat stripe rust by comparison of yields between varieties and years, page 886. Cold testing and retreatment of old corn seed are definitely advantageous, according to evidence reported by PAUL E. HOPPE, page 887. S. F. HASSAN reports studies on the pathogenicity of fungi causing root rots and seedling blights of oats, page 890.

Virus Diseases: H. H. McKINNEY concludes that protection and synergy tests with cereal mosaic viruses aid in determining relationships between viruses, page 898. Probably all bulbs of the Havemeyer hybrid lily carry a mottle virus complex, according to FRANK P. McWHORTER, page 904.

Vegetable Crop Diseases: EUGENE C. TIMS and RALPH T. BROWN discuss incidence of cabbage yellows in Louisiana in 1955, page 905. Evidence of transmission of Rhizoctonia solani in bean seed is presented by CHARLES M. LEACH and MERLE PIERPOINT, page 907.

The tobacco anthracnose fungus has a wide host range, according to results of cross inoculations reported by OMAR D. MORGAN, Jr., page 908.

EDDIE ECHANDI and R. H. SEGALL report the presence of the Ceratostomella canker of coffee trees in Costa Rica, page 916.

WILLIAM J. STAMBAUGH and CHARLES L. FERGUS conclude that ascospores of the oak wilt fungus remain viable over winter on insects contaminated from mycelial mats formed in the autumn, page 919.

"Results of the 1955 Fungicide Tests", page 923.

August weather, page 924.

Manuscripts for the REPORTER, page 925.

Vol. 40, No. 10--PLANT DISEASE REPORTER--Oct. 15, 1956

PARASITIC HERBACEOUS ANNUAL ASSOCIATED WITH CORN DISEASE IN NORTH CAROLINA

Howard R. Garriss and J. C. Wells[1]

A flowering plant tentatively identified as Striga sp. and associated with poor growth and dying of corn, Zea mays L., has been found in several fields in Columbus and Robeson Counties, North Carolina. As far as we have been able to determine to date, the occurrence of Striga spp. has not previously been reported in the Western Hemisphere (3).

In July 1956, samples of corn roots and soil from a problem field in Robeson County were submitted to the Plant Disease Clinic, North Carolina State College. Routine examinations of root and soil samples failed to reveal parasitic nematodes, fungi or bacteria which could account for the diseased condition of the corn. However, portions of fleshy, white and purplish underground stem-like structures bearing small scale-like leaves were found entangled among the corn roots. A graduate student from India, Mr. Akhtar Husain, assisting in the examinations, stated that these resembled the underground stems of Striga spp. which are reported to be parasitic on sugarcane in the Eastern Hemisphere (1, 2, 3, 4). Striga lutea Lour., commonly known as witchweed, has been recognized for many years as a serious parasite upon roots of maize in South Africa (1).

FIGURE 1. Corn field in Columbus County, North Carolina, approximately six weeks before harvest showing damage associated with the presence of Striga sp.

Subsequent visits were made to Robeson and Columbus Counties in response to a number of complaints of trouble in corn. In a number of corn fields in the area, an unknown plant bearing small pink flowers was found. In portions of fields where this plant occurred, corn was retarded in development. In some cases, the corn plants had died by the time they had reached a height of 3 to 4 feet (Fig. 1). In areas of fields where the plants were not present, corn exhibited normal development.

[1] In Charge, Plant Pathology Extension and Plant Pathology Extension Specialist, respectively, North Carolina State College, Raleigh, North Carolina.

FIGURE 2. Plants of Striga sp. showing close association of roots with those of corn and with above-ground parts adjacent to corn stalk.

FIGURE 3. Plant of Striga sp. with under-ground structures entwined about corn root (arrow).

FIGURE 4. Single plant of Striga sp. (left) in field where above-ground parts emerged from fringe area of corn (right) root system.

Underground fleshy stem structures similar to those previously examined in the laboratory were found entangled in the root systems of affected corn plants and could be traced to above-ground portions of the unidentified plants.

Plants were submitted to B. W. Wells, Emeritus Professor of Botany, who tentatively placed them in the genus, Striga. Leaf, flower and stem characteristics conformed closely to a description of this genus (1).

After careful washing of soil from affected corn root systems, masses of Striga roots were observed entangled among corn roots (Fig. 3). Microscopic examinations revealed the presence of numerous appressoria-like swellings on the fleshy, white roots of Striga at points of attachment to the yellowish corn roots. A similar association of Striga roots with sugarcane roots has been described (1). In the field, the above-ground parts of the Striga plants had emerged from the soil either adjacent to the corn stem (Fig. 2) or as far away as the corn roots extended (Fig. 4). Similar observations in the field and in the laboratory suggest that Striga may also be parasitic on crab grass, Digitaria sanguinalis (L.) Scop.

Striga spp. are annual plants (1, 3) which die after flowering and production of seed. Propagation is by means of the tiny seeds which are easily carried by the wind and by drainage and irrigation water (1). Fairly satisfactory control of the parasite in sugarcane by use of herbicides and rotation with non-susceptible hosts has been reported in Queensland (1, 3).

Further studies are underway.

Literature Cited

1. Bell, Arthur F., and W. Cottrell-Dormer. 1931. The cane-killing weed. Queensland Agr. Jour. 36: 463-473.
2. Cottrell-Dormer, W. 1924. Reports on "cane-killing" and "cane-stunting" weed. Queensland Agr. Jour. 21: 363 and 434.
3. Edgerton, Claude W. 1955. Sugarcane and its diseases. Louisiana State Univ. Studies, Biol. Sci. Ser., No. 3. Louisiana State Univ. Press.
4. Luthra, J. C. 1921. Striga as a root parasite of cane. Agric. Jour. India 16: part 5, 519-523.

PLANT PATHOLOGY DEPARTMENT, NORTH CAROLINA STATE COLLEGE, RALEIGH

INTERPRETATION OF RESULTS FROM A SOIL FUMIGATION TRIAL

J. G. Bald and R. N. Jefferson

Summary

1) A test of soil fumigation with Vapam against gladiolus diseases and pests was used as an example in discussing soil fumigation trials.

2) A pilot planting made before the main trial on the same site extended the range of information obtained.

3) The best estimate of mite populations was obtained from apparently normal corms in all treatments. Numbers were not necessarily a measure of the direct action of fumigant on the mites in the soil, because of the frequent association of mites with disease.

4) A simple rating method for estimating the severity of bacterial scab on corms provided an estimate of infection roughly equivalent to a square root transformation of the percentage of corms obviously infected.

5) Cormels dipped in New Improved Ceresan and planted in soil given an intermediate dosage of Vapam were severely infected by scab; those in soil with a lower dosage were less severely infected. This was interpreted as due to the relative effect of treatments on the scab organism and its antagonists.

6) Fusarium infection decreased evenly with increasing Vapam dosage.

7) A crude mathematical model was formulated for the relation between fumigant dosage and infection of test plants grown in treated soil. The cumulative action of fumigant on the soil-borne pathogen population, and of the residual pathogens on the test plants were calculated to give an asymmetrical sigmoid relation between dosage (log dose) and percentage infection.

8) Some consequences of this type of relationship were found to explain what are often accepted as anomalous results of fumigation.

INTRODUCTION

A recent trial of fumigation with Vapam in a field soil, to control both pests and diseases of gladiolus, illustrates some problems of interpreting results from such experiments. The layout of the experiment is described and the data are summarized elsewhere (6).

Vapam (sodium N-methyl dithiocarbamate dihydrate) is a general pesticide which has some action on insects, mites, nematodes, fungi, bacteria, and higher plants (weeds). It represents a class of soil fumigants which will be presented for trial in increasing numbers to plant pathologists, nematologists, and entomologists. It will be difficult and in many instances undesirable to test their action in the field against single organisms. The complexity of trials will increase, but the opportunities for discovering new and interesting facts will be multiplied.

The Vapam trial was made on a grower's farm in a heavily infested soil, which had carried planted or volunteer gladiolus crops several years in succession. There were untreated checks and 4 rates of application, 215, 430, 860, and 1720 lb. of Vapam per acre; 3 single applications from 215 to 860 lb., and 3 double (split) applications totalling 430-1720 lb. Treatments were replicated 4 times in randomized blocks; single and split applications were grouped separately in 2 adjacent areas. There were 5 lots of planting material in each plot: Spotlight corms dipped in New Improved Ceresan before planting, Spotlight corms not dipped, Spotlight cormels not dipped, Evangeline cormels dipped and not dipped.

USE OF PILOT PLANTING

From a grower's viewpoint, the crucial data obtainable from a fumigation trial on infested field soil are the yield and quality of the saleable parts of crops, planted in the fumigated as against untreated soil. Often the experimenter would rather know directly the reduction, induced by treatment, in the quantity of soil pests or pathogens and the capacity of any residual infestation to increase again in the soil. Techniques to obtain such information directly are scanty and often troublesome to apply. As a substitute, healthy plants are set in treated and untreated soil, and used as traps for the pests and pathogens, and as indicators of treatment effects.

Interpretation of the results obtained from the test plants is a problem which the experimenter may sometimes attempt to solve by taking additional data. In the Vapam trial, an un-

replicated strip planting of gladiolus corms was made through all treatments on the edge of the split application experiment soon after the first (September) fumigation. This planting, made months before the main trial began, revealed the presence of mites, probably Rhizoglyphus rhizophagus Banks; two pathogens previously thought to be present, Stromatinia gladioli Drayton and Fusarium oxysporum Schlecht f. gladioli Snyder & Hansen; and a significant infestation of the soil with the bacterial scab organism, Pseudomonas marginata (McCull.) Stapp. However, its greatest value was that it demonstrated the changing balance of pathogens with the change of seasons. Stromatinia produced symptoms on the new corms during the cooler months, but not during the summer. During the summer, bacterial scab and Fusarium were more severe on the new corms. This seasonal response of the pathogens reduced the efficiency of the host plants as indicators of treatment effects; but as final arbiters of the commercial value of Vapam for gladioli, they indicated that fumigation might increase yields during different seasons of the year. The pilot planting was of more value and was far less trouble than an additional trial on another area of soil.

The same general principles of design apply to trials restricted to one growing period. Extra plantings, plots, or rows within plots may be made for collecting supplementary data, or for providing soil samples from the root zone of the growing plants; areas within plots may be left bare, or periodically planted with trapping hosts, to obtain additional information on soil infestation. The whole subject of supplementary data is relatively unexplored, and will repay detailed examination.

QUANTITATIVE RECORDS

Two problems of record-taking are illustrated by the Vapam trial. One (a) arose from the question of how to sample the mite population, the other (b) from the nature of the units in which bacterial scab was recorded.

(a) -- Mites of the species represented in this trial invade healthy as well as diseased tissues, but appear in greater numbers when lesions are present (6). Fumigation could reduce the mite population directly or by reducing disease. In the pilot planting, counts were made on both healthy and diseased corms. The latter bore a higher population of mites. In the main trial the healthiest available daughter corms growing from the planted Spotlight corms were chosen for the mite counts. A significant reduction of mites in the fumigated plots was recorded. These counts were the best indication available that fumigation reduced the mite population in the soil, but they cannot be accepted as unassailable evidence of direct action. Indeed, in this trial there was a degree of association between the size of the mite population on apparently healthy corms and the average severity of scab in the plots from which they were dug. This suggests a more rapid multiplication in diseased tissues, and some migration to healthy corms, perhaps by way of intertwining root systems. In future trials of this kind more exact information might be gathered by planting, on the fumigated and untreated soil, additional plots of a second host plant favored by the mites, but immune from the diseases present in the soil. Parallel mite counts on this host and on gladiolus should allow distinction between the direct and indirect action of the fumigant.

(b) -- Infection with bacterial scab was estimated, after harvest, on corms grown from the cormels. These included equal numbers of Evangeline dipped in New Improved Ceresan, Evangeline not dipped, and Spotlight not dipped. Daughter corms infected with bacterial scab were classified as mummies, and rated 3; as severe infection, 2; and as mild or commercially negligible infections, 1. Healthy corms were rated 0. The mean rating for composite samples of 60 corms per plot, which included 20 from each cormel planting stock, was taken as the estimate of infection. Such a weighted mean lies half-way between the proportion of plants infected and the mean number of lesions per corm.

A comparison was made between (a) the number of corms in each lot of 20 which were commercially undesirable because of scab -- mummies plus severely diseased -- and (b) the mean ratings for the same samples. Plotted on a log log scale with numbers of corms on the y axis, the points fell on a line of slope 2, i. e., the square roots of the numbers of severely infected plants were proportional to the mean ratings. The data for individual lots of 20 corms are in Figure 5, plotted not as logs, but on an arithmetic scale. The curve derived from the straight line of slope 2 is inserted to show the goodness of fit. Functionally, the ratings were equivalent to a square root transformation of the numbers of severely infected corms. In

general, a square root transformation reduces erratic variation of means, and equalizes variances of high and low values. It is applicable to counts of insects or other organisms which multiply in groups or aggregates. Bacterial scab lesions increase by widespread re-infection on individual corms, and the local environment appears to encourage or check this process in a marked degree. Therefore the ratings, because in this instance they were equivalent to a square root transformation of the quantal data, were about the best simple means of recording degrees of infection.

DOSAGE EFFECTS

1) Bacterial Scab: In the single application experiment, increasing dosages of Vapam were associated with a fall, a rise, and a second fall in the severity of bacterial scab (6). This deviation from a simple relationship and also some irregularities in the ratings from the split application experiment were traced to the effects of the preplanting dip, New Improved Ceresan. They are illustrated in Figure 6. The explanation offered was that the residual action of the New Improved Ceresan plus the higher dosages of Vapam caused the failure or erratic development of a protective microflora in the rhizosphere of the growing plants. Traces of scab inoculum in the soil were thus enabled to produce numerous, serious, but somewhat erratic infections. Description of a similar action of phenyl mercury acetate on damping-off of pine seedlings has been published since this was written (4).

It is normal to overlook the possibility that erratic results from field trials may have real significance, but the mere fact that one set of results is more variable than another may be a clue that the experimenter is seeking. There are several simple statistical procedures which provide means for comparison and judgment of such treatment effects. Where increasing dosages of a soil fumigant produce responses like those of bacterial scab in the single application experiment, a form of analysis described by Cochran and Cox (2) is applicable. If data from untreated checks and 3 increasing dosages of fumigant are labelled T_1, T_2, T_3, T_4, the comparison $T_1 + T_2$ versus $T_3 + T_4$, with 1 degree of freedom, will test the significance of an increasing action of the fumigant with increasing dosage. The comparison $T_1 + T_4$ versus $T_2 + T_3$ will test for a maximum or minimum value at intermediate dosages. The comparison $T_1 + T_3$ versus $T_2 + T_4$ will test the possibility of 2 maxima and minima, as in the case of the ratings for bacterial scab. Whether any practical significance is attached to the statistical significance of these more complex relationships must depend on their inherent likelihood. In the present instance, a complex reaction seems reasonable, and the risk of losing information by ignoring the erratic figures for bacterial scab seems more serious than the risk of merely rationalizing an extreme example of random variation.

2) Fusarium Disease: The presence or absence of Fusarium lesions on the same samples of 60 corms per plot was recorded. The number of separate lesions on each corm was small, often one only. Fusarium lesions expand rather than increase in numbers from reinfection of underground plant parts, and a single lesion may destroy a whole corm. The number of plants with lesions was more closely related than in the case of bacterial scab to the number of foci at which invasion occurred from the soil. A potential variable, more likely than reinfection to influence the relationship between lesions and invasion foci, was the proportion of unobserved cases in which the organism, after penetrating the outer scales or a root base, may have failed to form a visible lesion in the tissue of the corm. Experience has shown that incipient Fusarium lesions may become manifest during storage. As some time was allowed for the development of lesions before counts were made, only latent infections and very small lesions were missed.

Among the checks complete destruction of many corms severely infected by Fusarium made a consistent count impossible. Results for all dosages of the single and split applications of Vapam are summarized in Figure 4. Dosage is plotted on a log scale, and percentage infection on an arithmetic scale. The result approximates to a straight line relationship. There is no indication of a complex reaction to dosage similar to that of bacterial scab. Further discussion of the Fusarium type of relationship appears in the following section.

PERCENTAGE OF DISEASED HOST PLANTS AS A MEASURE OF PESTICIDAL ACTION

Probit analysis is the established method of obtaining essential information from dosage mortality data. Analysis of dosage-infection data is the fundamental problem in evaluating soil fumigation trials, but the probit method (1, 3) is not automatically applicable to the results of

trials in field soils, particularly when susceptible plants are used as indicators of the lethal action of the fumigant. The lack of homogeneity in soil, and its resistance to penetration by the fumigant, are added to the natural variability of the organisms subjected to fumigation. Even if the parasitic organisms should be sampled directly, the normal relationship between dosage and mortality (3) is likely to be distorted. Also when susceptible plants are used as indicators, the number infected or infested is unlikely to be directly proportional to the population of parasites, unless the number of plants affected is less than 20 percent of the number sampled. If the probability of infection may be derived from the Poisson series (see below) about 69 particles of inoculum will be involved in infection of 50 plants out of 100, and 400 particles will be needed to infect 99 out of 100.

To discover what modifications of scale and transformations of the quantal data might enable the use of probits or the discovery of a substitute method of evaluation, it is necessary to outline some of the consequences of making fumigation tests in field soil, and using plants as indicators. The following crude approximation indicates the type of relationship which may be expected.

In soil a fumigant is likely to diffuse with varying speeds and reach widely different concentrations in larger and smaller pore spaces; and it may be dissolved, adsorbed, or absorbed. In all these ways it may reach and react on the organisms in the soil, or alternatively it may be lost or rendered inactive. The net effect is likely to extend the range of dosages that are less than 100 percent lethal, and to skew the mortality dosage (log dose) frequency curve (Fig. 2), as well as the mortality dose frequency curve (Fig. 1), in which dose is plotted in arithmetic units (3).

As practically no evidence is known to the writers which would help more accurately to define the frequency curve of survival against doses of a fumigant in field soils, a beginning is made from the generalization of Horsfall (5) that some form of the law of diminishing returns applies to the action of increasing doses of a fungicide (or more generally of a pesticide). It is assumed that a moderately effective dose, giving perhaps 30 percent kill, will include the rise and peak of the mortality dose frequency curve (Fig. 1), and that subsequent increments of fumigant, representing increasing concentrations, will kill always the same proportion of organisms, although not necessarily the same proportion as the initial dose, d. Zero dose is left out of account, i. e., untreated checks do not enter into the calculations. Suppose the dose 2d kills one-third the organisms that would have remained if dose d only had been applied, dose 3d in addition kills one-third of the remaining two-thirds, and so on.

In general, if y is the number of organisms alive after fumigation,

$$y_{\kappa} = y_d(1 - K_d)^{\frac{\kappa}{d}} \qquad (1)$$

κ being a dose of fumigant greater than d. The value K_d is the fraction of the remaining organisms killed with each additional increment, d, of fumigant applied. Suppose d is taken as the unit of dose, e.g., d = 200 lb. of the fumigant per acre = 1 unit; κ can then be given in terms of these units; e.g., for a dose 3d = 600 lb., $\kappa = 3$.

Suppose the unit dose, d, reduces the number of organisms per unit area of soil from 130 to an average of 92.5. The latter figure is then the reference point from which all calculations are made. The equation becomes,

$$y_{\kappa} = y_d(1 - K_d)^{\kappa - 1} \qquad (2)$$

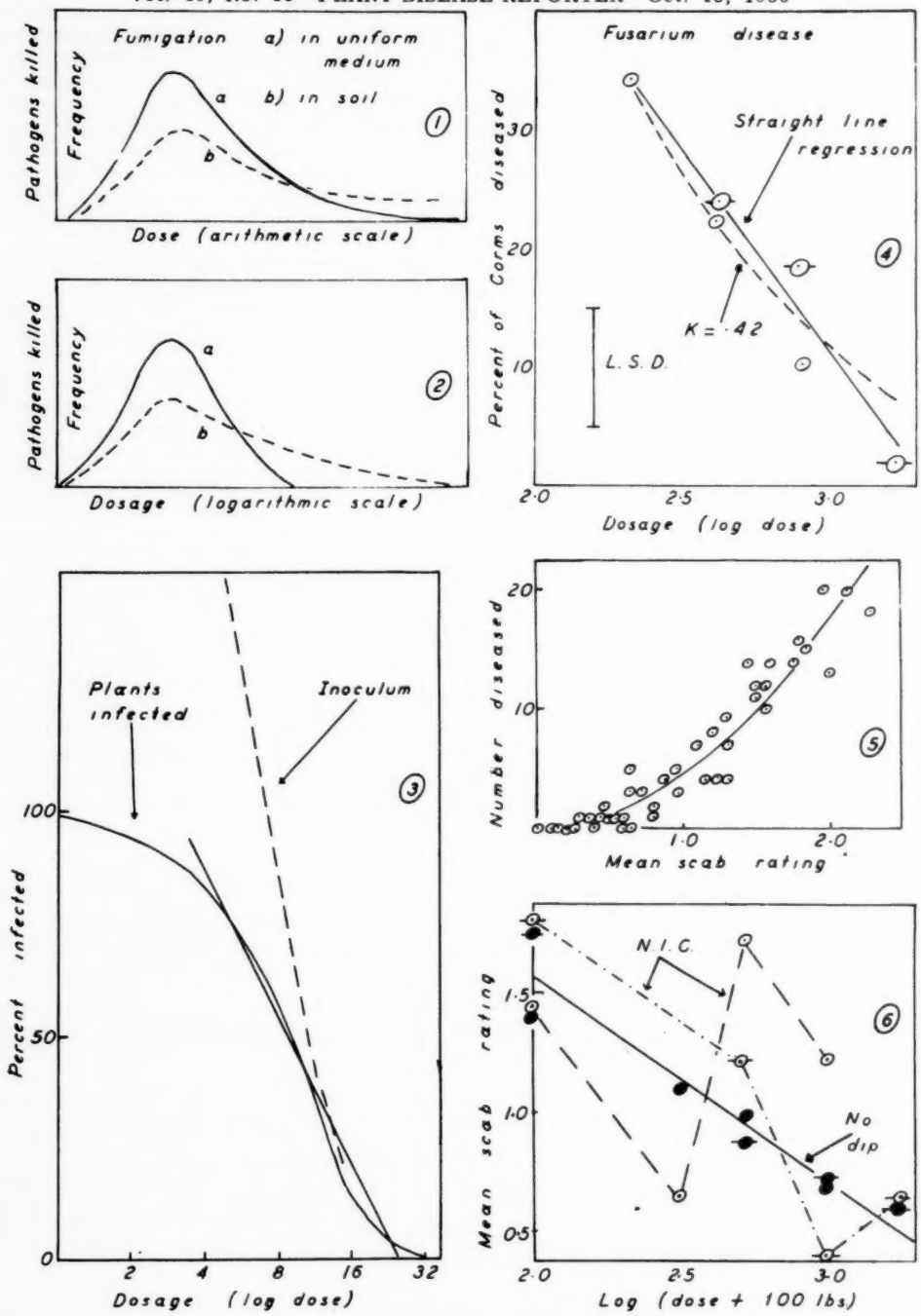
Dose $\kappa = 4$ is applied to equivalent plots of soil. The increment of dose in units of d is $\kappa - 1 = 3$. This increment reduces the organisms to 20 per unit area of soil (y_{κ}). Converting to logs,

$$\log_{10} y_{\kappa} = \log_{10} y_d + (\kappa - 1)\ (\log_{10} \overline{1 - K_d})$$

$$1.301 = 1.966 + 3(\log \overline{1 - K_d})$$

$$1 - K_d = .60$$

$$K_d = .40$$



FIGURES 1-5. 1--Contrast between a) kill of an organism by increasing doses of fumigant in a homogeneous medium, b) kill of the same organism as it is assumed to occur in soil which is not homogeneous. 2--Same as Fig. 1 except dose is converted to a logarithmic scale (dosage). 3--Postulated relationship between percent plants infected in soil previously treated with increasing dosages of fumigant. Based on curve b, Fig. 2 and a random distribution of particulate inoculum in the soil. 4--Percent of 120 gladiolus corms sampled diseased with Fusarium, taken from soil

Each increment of dose, d, reduces the number of organisms by 40 percent of the value that would have been reached by the previous increment. If $\int = 3$ is considered as a single increment, i.e., the unit of dose is 3d:

$$1.301 = 1.966 + \log (1 - K_{3d})$$

$$K_{3d} = .784$$

An answer is given in terms of the proportion of organisms inactivated, which is not determined directly in the ordinary fumigation trial. To link it with the number or proportion of plants infected, use is made of a relationship derived from the Poisson distribution. This relationship provides a first approximation for the probability of obtaining infection when various amounts of particulate soil-borne inoculum invade the test plants and produce evident infections (unpublished data). The estimate of this probability is $\frac{i}{N}$, which is the number of host plants infected divided by the maximum number of plants submitted to infection and capable of being infected. If all the plants are about equally susceptible, and none is protected by an adventitious mechanism, such as an impermeable microflora in the rhizosphere, then N is all the plants set in the treated soil. As infection is at random, some plants will be infected 2, 3, or more times, while others are not infected. The formulation gives

$$\frac{i}{N} = 1 - e^{-m}, \qquad (3)$$

where m is the mean number of units of particulate inoculum effective in causing 1 of the i infections. m is fractional, and the denominator of the fraction is N, the maximum, not i the number infected. Also m is directly related to the total inoculum, but appears to be a very small part of it (7). $m = cy$, where y, the number of inoculum particles in a unit of soil, may be very large, and c, the proportion of them actually causing infection, is very small.

Equation (3) above may be put in the form,

$$m = cy = \log_e N - \log_e (N - i) \qquad (4)$$

Modifying equation (2) to evaluate the reduction by a soil fumigant of the effective inoculum, or that proportion of it infecting the $i_{\int}$ plants, we have

$$(1 - K)^{\int - 1} = \frac{\log_e N - \log_e (N - i_{\int})}{\log_e N - \log_e (N - i_d)} \qquad (5)$$

Reducing i_d and $i_{\int}$ to percentages:

$$\log_e (N - i_{\int}) = 4.60517 - 4.60517(1 - K)^{\int - 1} + \log_e (N - i_d)\ (1 - K)^{\int - 1}$$

The dose $\int$ is known, $i_{\int}$ and i_d are determined experimentally, and K may be calculated.

The form of this relationship is shown graphically in Figure 3, where the percentage of the N plants infected is plotted against dosage, i.e., dose on a logarithmic scale. It is an asymmetrical sigmoid curve. The complementary curve, showing percentage healthy, would also be asymmetrical and sigmoid. Therefore the probit transformation could not be used directly for data in this form. On the other hand, the curve between 10 and 80 percent infection differs so little from a straight line that it is doubtful whether data from a field trial would normally reveal its curvature. The straight line relationship obtained in the Vapam trial

---

(Figures cont.)

submitted to increasing dosages of Vapam. Data fitted with a straight line and with the relationship illustrated in Fig. 3 (hatched line). 5--Relationship between mean scab rating of sampled gladiolus corms for individual plots and number obviously diseased. Data fitted with curve transposed from straight line relationship between log rating and log number obviously diseased. 6--Mean scab ratings against log (dose + 100 lb.) to show erratic variation of infection with increasing dosage when cormels dipped in New Improved Ceresan were planted in treated soil. Solid dots, not dipped; circles, dipped. A straight line was fitted to the data for the undipped series.

discussed above, between numbers of gladiolus corms (plants) visibly infected with Fusarium disease and log dose is not discordant with this formulation (Fig. 4). The curve for K = .42 fits the points within the limits of the experimental error. If the same formulation is found to give adequate approximations for other field trials and other fumigants, data may be presented conveniently as percentage infection against dosage. If the trials are concerned with dosages of fumigant producing infections over 80 percent, or if extreme reductions of inoculum are being tested, calculation of K may be advisable.

Using this formulation it will be possible to calculate and present values of K which will be (theoretically) independent of the level of infection at which they are estimated. K may be used under similar conditions to measure the efficiency of different fumigants, or of the same fumigant acting on different organisms, or it may be used to compare the action of one fumigant on the same set of organisms under different conditions. Also the formulation defines and in that sense explains a number of facts otherwise denied consistent quantitative expression.

It has been found by hard experience that moderately efficient soil treatments are often useless when applied to heavily infested soils. The reason for this is plain when data are examined by substitution of appropriate values in the above equations. Suppose, as sometimes happens with Fusarium disease of gladiolus, the soil inoculum causes nearly 100 percent infection. For simplicity the initial dose, d, is taken as having the same efficiency as succeeding increments of dose. Suppose infection in the checks is 98 percent, and Vapam used at 200 lbs. has the same efficiency as in this trial, i.e., K = .42. Application of 200 lbs. will reduce infection among the host plants only to 88 percent. If a fumigant with efficiency K = .90 had been used, the reduction would have been to 30 percent. When soil infestation is high only an efficient fumigant is worthwhile. When soil infestation is kept low by cultural means or regular treatment, the use of a less efficient soil treatment may give profitable results.

It is sometimes found that normal doses even of an efficient soil treatment fail when infestation is very high. The question arises whether an increase in dosage might have been justified. Suppose the initial dose reduced infection of susceptible host plants from practically 100 percent only to 95 percent, but it was known that the material had given a 90 percent kill of inoculum in other trials. Doubling the dose should have reduced infection by an additional 70 percent to 25 percent. If this expected gain more than balanced the cost of applying the double dose of fumigant, it would have been well worth considering.

Many other experiences fall into place and find an explanation when considered from this point of view. It is to be expected that others will not fall into place, because the assumptions made in obtaining the equations are enormously simplified and may be partly incorrect. An adequate formulation, however, still will have to take account of several established facts, such as the radical increase in units of soil inoculum needed to infect the last 5 percent of plants compared with the first 5 percent. Insofar as these facts are correctly evaluated by the present formulation, the general conclusions reached by using it will agree with more accurate models of the system, and therefore will approximate to field experience.

Literature Cited

1. Bliss, C. I. 1935. The calculation of the dosage mortality curve. Ann. Appl. Biol. 22: 134-167.
2. Cochran, W. G., and G. M. Cox. 1950. Experimental Designs. John Wiley and Sons, Inc. New York.
3. Finney, D. J. 1947. Probit Analysis. Cambridge University Press.
4. Gibson, I. A. S. 1956. An anomalous effect of soil treatment with ethylmercury phosphate on the incidence of damping-off in pine seedlings. Phytopathology 46: 181-182.
5. Horsfall, J. G. 1945. Fungicides and Their Action. Chronica Botanica Co. Waltham, Massachusetts.
6. Jefferson, R. N., J. G. Bald, F. S. Morishita, and D. H. Close. (In press). Effect of Vapam on Rhizoglyphus mites and gladiolus soil diseases. Jour. Econ Entom.
7. Leach, L. D., and A. E. Davey. 1938. Determining the sclerotial population of Sclerotium rolfsii by soil analysis and predicting losses of sugar beets on the basis of these analyses. Jour Agr. Res. 56: 619-663.

DEPARTMENT OF PLANT PATHOLOGY, AND DEPARTMENT OF ENTOMOLOGY, UNIVERSITY OF CALIFORNIA AT LOS ANGELES

RESULTS OF LABORATORY EXPERIMENTS WITH VAPAM FOR CONTROL OF NEMATODES

Bert Lear

SUMMARY

In a series of greenhouse experiments, Vapam solutions sprinkled on the surface of flats of infested soils eradicated root-knot nematodes at the rate of 1/2 pound/100 sq. ft. A gas-proof cover for the soils did not appear essential. Additional experiments demonstrated that Vapam applied as a concentrate solution to surface soil is retained in the surface of wet soils and is carried out of the surface layers of dry soils by addition of water. Most consistent results were obtained by applying the entire dosage as a water drench or by applying a concentrated Vapam solution before and following the application of water. Diffusion patterns of a 4 ml dose of Vapam showed this amount diffused 6 to 8 inches laterally but only 4 inches or less vertically.

Many new problems were encountered with the introduction of Vapam (sodium methyl dithiocarbamate) as a commercial treatment to control nematodes. Information was needed concerning the movement of Vapam in soil since this material is water soluble. Vapam has been most effective when applied in water as a flood or overhead sprinkler treatment, but little is known about its dispersion in soil whether applied by these methods or by conventional soil injection methods.

EXPERIMENTAL PROCEDURE AND RESULTS

Flat Treatments

Two experiments were conducted to determine whether a successful application of this material could be made to flats of infested soil for control of root-knot nematodes, Meloidogyne incognita var. acrita. Such a treatment would be especially useful to nurserymen. In the first experiment, three rates of application were tested in a series of flats covered with polyethylene following treatment and in another series in which no cover was used. The covers were left on the flats for 5 days following application of the chemical. All treatments were applied in 500 ml water per flat, barely wetting the surface of the soil. The flats used were 20 x 14 x 2-1/2 inches in size, approximately 2 square feet. Rates applied per flat were 4.75, 9.5, and 19.0 ml, equivalent to approximately 1/4, 1/2, and 1 pound/100 sq. ft.[1]. Soil samples were removed from the four corners, the two sides, and center of the flat 7 days after treatment. These samples were thoroughly mixed and a 1-pint sample removed to a 4-inch clay pot, to which a tomato seedling was transplanted. Gall counts on plants removed after 4 weeks indicate that all dosages, whether covered or not, were very effective, with no nematodes being recovered on indicator plants. Untreated samples contained 65 to 241 nematodes per pint of soil.

It seemed apparent that a cover for soils treated in flats was not necessary at the dosages used in the first experiment. In the second experiment, rates of application were decreased and soil moisture varied at time of treatment as were the methods of application. Vapam was added in a concentrated solution as in the first experiment to both relatively dry and wet soils. In other series of tests, various amounts of water were applied to the soil following treatment to determine the role of water in the distribution of Vapam in soil. The wet soil series consisted of the original soil which was saturated with 1500 ml of water. This amount was found necessary to saturate the entire soil mass. Vapam treatments consisted of 2.4 ml and 4.75 ml per flat. These were applied in 500 ml of water as in the first experiment. In one series, an additional 250 ml of water was sprinkled on the surface following treatment and

---

[1] 31% sodium N-methyl dithiocarbamate (anhydrous) which is equivalent to 3.8 lbs. dihydrate per gallon.

Table 1. Results of Vapam treatments applied to flats of infested soil for control of root-knot nematodes.

Treatment	Dosage (ml. flat)	Soil moisture at time of treatment	Surface seal after treatment	Mean no. galls/pt. soil Flat location where samples removed Center	Sides	Corner
Check	0	Dry	None	76[e]	47	49
Check	0	Wet[a]	None	40	40	41
Vapam	2.38[b]	Dry	None	0	0	0
Vapam	2.38	Wet	None	0	12	10
Vapam	2.38	Dry	Sprinkled[c]	0	0	0
Vapam	2.38	Wet	"	0	0	0
Vapam	2.38	Dry	Saturated[d]	0	0	0
Vapam	4.75	Dry	None	0	0	0
Vapam	4.75	Wet	None	0	0	0
Vapam	4.75	Dry	Sprinkled	0	0	0
Vapam	4.75	Wet	"	0	0	0
Vapam	4.75	Dry	Saturated	0	0	0

[a]1500 ml. water applied per flat before treatment.

[b]Applied in 500 ml. water - adequate to cover surface.

[c]250 ml. water sprinkled on surface after treatment

[d]1000 ml. water applied after treatment.

[e]Large gall masses in all check plants, tops and roots severely stunted.

Table 2. Gall counts on tomato plants grown in soils removed at various depths in column of soil which had been treated with Vapam solutions applied at the surface.

Depth in column from which soil samples removed (inches)	Check	Mean no. galls/tomato plant Vapam 0.5 ml	Vapam 1.0 ml	Vapam 2.0 ml
1	Plants killed by nematodes	dead	58	34
7	" " " "	70	26	dead
12	" " " "	79	42	dead
18	" " " "	73	63	39
24	" " " "	34	68	7
30	" " " "	90	dead	52
36	" " " "	72	50	87
42	" " " "	96	37	36

in yet another series, 1000 ml of water was added to saturate the soil following treatment. Soil was removed from the locations in the flat as in the first experiment and tomato seedlings transplanted into the samples. Gall counts (Table 1) indicate that all treatments except the lower dosage of Vapam (2.4 ml), applied as a concentrated solution to the surface, eradicated nematodes. It appears that if this chemical is added to saturated soil, it is retained in the surface unless additional water is added to carry it throughout the soil mass. The same dosage applied to a drier soil eradicated nematodes present.

Soil Column Treatments

Four experiments were conducted to determine the downward movement of Vapam solutions applied to the surface of the soil. In the previous two experiments it was shown that such solutions moved at least 2 1/2 inches when applied as a surface application. In the first experiment, dosages of 0.5, 1.0, and 2.0 ml of Vapam per container were applied. These rates are equal to 12.5, 25, and 50 pounds per acre, respectively. The solutions were applied in 25 ml to the surface of soils contained in a column made of cheese cans having a surface measurement of 4 x 3 inches and a depth of 11 inches. Four of these cans with the bottoms removed were taped together with masking tape to form the column. Root-knot infested soils of 50 cc quantities were tied in cheesecloth bags and buried at intervals in the soil column. After application of the chemical, enough water was added to keep the soil surface moist. Ten days after treatment, the soil samples were removed and placed in clay pots of fumigated soil to which tomato seedlings were transplanted. Gall counts made 4 weeks later (Table 2) indicate that few nematodes were killed in the columns at the dosages used for this experiment.

In the second experiment, the same soil columns were set up, the entire column being filled with infested soil. Dosages of 2 ml and 4 ml of Vapam were applied to the columns in the following manner:

1. 100 ml Vapam solution followed by 1100 ml water.
2. 1100 ml water followed by 100 ml Vapam solution.
3. 1200 ml Vapam solution.

After one week, soil samples were removed at 6-inch intervals and placed in clay pots to which tomato seedlings were transplanted. Gall counts made one month later (Table 3) show extreme differences in treatment efficacies. The Vapam was apparently retained in the few inches near the soil surface if the soil was saturated prior to treatment. If the Vapam solutions were followed by applications of a considerable amount of water to dry soil, the Vapam was carried past the surface so rapidly, or diluted so much, that it was ineffective. Vapam penetrated the soil to 14 inches but not to 21 inches when the soil column was saturated with Vapam solutions.

In a third test, Vapam solutions of higher concentrations were applied to the soil columns by adding the solution to the surface followed by 500 ml water. Gall counts on tomatoes grown in soil samples removed from the columns (Table 4) indicate again that the chemical was moved from the surface soil where nematodes survived. Nematodes recovered from the 12-inch level were killed but not at the 24-inch level.

In a fourth similar experiment, cheesecloth bags of soil infested with root-knot nematodes were buried in the metal columns at 6-inch intervals. Vapam was applied to the surface of the soil columns as previously described except that the amount of water utilized was 1800 ml in an attempt to drive the Vapam deeper. In addition, a split application of Vapam concentrate was included in the dry soil series to determine whether nematodes missed in the surface could be killed by the application of additional toxicant. Gall counts on tomatoes (Table 5) confirm previous results on movement of Vapam in the column. The split-application resulted in eradication of nematodes in the surface.

Diffusion of Vapam Applied by Injection

An experiment was conducted in a 32-gallon container of soil to check on the vertical and lateral diffusion patterns of a given amount of concentrated solution applied as a single injection application. Fifty-six cheesecloth bags each were filled with a sandy loam infested with root-knot nematodes and with soil infested with cysts of the sugar beet nematode, Heterodera schachtii. The bags were buried at various intervals from point of injection of the chemical. A dosage of 4 ml of Vapam, equivalent to approximately 40 gallons or 160 pounds of Vapam per acre, was applied 8 inches deep to the drum of soil. The drum was left uncovered in the

Table 3. Root-knot gall counts from tomatoes grown in soil samples removed from soil columns to which Vapam had been applied by various methods.

Depth at which soil samples removed (inches)	Mean number nematode galls/plant						
		Vapam Surface Appl.		Vapam Surface Appl. followed by water		Vapam Saturated Soil Surface Appl.	
	Check	2 ml	4 ml	2 ml	4 ml	2 ml	4 ml
1	144	16	10	206	246	0	0
7	-	0	0	0	103	120	188
14	-	0	11	0	0	140	50
21	-	46	132	0	0	92	32
27[a]	-	106	117	139	120	69	93
33	-	128	147	122	129	42	109

[a] Water used in treatments penetrated to this depth, below was relatively dry.

Table 4. Gall counts from tomatoes grown in soil samples removed from various locations in a soil column treated with Vapam solutions.

Depth at which soil samples removed (inches)	Mean number galls/plant			
	Check	Vapam[a] 2 ml	Vapam[a] 4 ml	Vapam[a] 8 ml
1	53	89	80	104
12	-	96	0	0
24	-	78	83	300
36	-	dead	dead	dead
48	-	dead	dead	dead

[a] Vapam concentrate applied to soil surface in 100 ml followed by 500 ml of water.

Table 5. Gall counts from tomatoes grown in soil samples removed from soil column to which Vapam had been applied by various methods.

Depth at which soil samples removed (inches)	Mean number nematode galls/plant							
	Check	Drench to[a] dry soil		Concentrate to[b] wet soil followed by water		Concentrate to[c] dry soil followed by water		Split application[d] concentrate to dry soil
		2 ml	4 ml	2 ml	4 ml	2 ml	4 ml	4 ml
1	170	0	0	0	2	135	7	0
6	-	0	2	0	132	49	0	0
12	-	0	3	125	45	3	0	0
18	-	0	7	88	69	2	0	0
24	-	76	5	58	160	0	0	0
30	-	66	154	139	142	104	-	0
36	-	93	147	85	187	50	54	177

[a] 2ml and 4 ml Vapam with water to make 1800 ml.

[b] 1700 ml water followed by 100 ml concentrate containing 2 ml and 4 ml Vapam

[c] 100 ml concentrate containing 2 and 4 ml Vapam followed by 1700 ml water

[d] 100 ml concentrate containing 4 ml Vapam split with 75 ml applied prior to adding 1700 ml water followed by 25 ml Vapam concentrate.

Table 6. Number nematodes surviving in 50 cc soil samples recovered from various locations from point of injecting 4 ml of Vapam.

Vertical distance (inches)	Root-knot Nematode: Lateral distance from point of injection (inches) 8	6	4	2		2	4	6	8
1	64	245	244	0		0	174	85	127
4	0	0	0	0		0	0	0	123
8	0	0	0	0	x	0	0	0	0
12	0	0	0	0		0	0	117	475
16	90	63	97	205		132	132	73	131
20	98	70	35	45		92	90	59	72
24	8	59	43	115		66	20	47	9
Vertical distance (inches)	**Sugar Beet Nematode: Lateral distance from point of injection (inches)** 8	6	4	2		2	4	6	8
1	15	15	50	0		0	5	7	32
4	25	0	0	0		0	11	5	18
8	0	0	0	0	x	0	0	1	73
12	0	16	1	0		3	5	1	95
16	19	4	12	24		2	9	15	38
20	0	0	2	1		0	3	12	9
24	0	7	3	40		0	40	3	8

[x]denotes point of application of chemical.

headhouse where the soil temperature remained at 78°F. After 7 days the bags were removed from the container and added to pots of fumigated soil, in which either tomato seedlings or sugar beet seeds were planted. Counts of galls for the root-knot series were made after 4 weeks and female counts of sugar beet nematode made after 6 weeks. Data (Table 6) indicate that lateral movement of this amount of Vapam extended for 6 to 8 inches. Vertical diffusion from point of injection did not appear much more than 4 inches. Nematodes present in the surface inch of soil survived except for those immediately over the injection.

DEPARTMENT OF PLANT NEMATOLOGY, UNIVERSITY OF CALIFORNIA,
DAVIS, CALIFORNIA

A PRELIMINARY REPORT ON SOIL FUMIGATION FOR CONTROL OF PARASITIC NEMATODES ON CORN AT CURTIS, LOUISIANA

J. Y. Oakes, C. N. Bollich, D. R. Melville,
M. J. Fielding, and J. P. Hollis

Nematode surveys conducted in the corn-producing areas of Louisiana have revealed the presence of large numbers of parasitic nematodes about and in the roots of corn. Three principal parasitic nematodes were found to be widely distributed: two ectoparasitic forms (Tylenchorhynchus sp. and Trichodorus sp.) and one endoparasitic form (Pratylenchus zeae, Graham 1951). P. zeae was found to be most widely distributed, occurring in 75 percent of the survey samples.

A soil fumigation experiment for control of parasitic nematodes on corn was initiated in February, 1955 at the Red River Valley Experiment Station, Curtis, Louisiana. The site chosen for the test was on a Yahola very fine sandy loam, a highly productive alluvial soil of the Red River Valley. Large numbers of P. zeae, Tylenchorhynchus sp., and Trichodorus sp. were found in pre-fumigation soil samples.

Two nematocides and one nematocide-fungicide were applied February 15, 1955 at the rates indicated in Table 2. The physical condition of the soil and the temperature were ideal for soil fumigation. Good nematode control was obtained with "Dowfume MC2" and "D-D mixture" but not with "Dowfume W-85" [1]. The "D-D mixture" and "Dowfume W-85" were applied with a MacLean fumigun at a depth of 6 inches, using 12-inch centers. "Dowfume MC-2" was applied under polyethylene covers, which were removed after 48 hours. The fumigants were used at a high rate to insure control of the parasitic nematodes. The experimental design was a randomized block with four replications. Each plot was 14 feet wide by 50 feet long and there was a 4-foot buffer zone between plots.

Sixty pounds of nitrogen per acre in the form of anhydrous ammonia was applied March 10,

Table 1. Numbers of parasitic nematodes based on 1-pint samples of soil. (Each value is the average for 4 plots)

Nematodes	Date of Sampling		
	Mar. 1, 1955	May 3, 1955	June 3, 1955
Check			
Tylenchorhynchus sp.	600	100	300
Trichodorus sp.	16	0	60
Pratylenchus zeae	45	120	50
Dowfume MC-2			
Tylenchorhynchus sp.	0	7	50
Trichodorus sp.	0	0	30
P. zeae	0	0	10
D-D			
Tylenchorhynchus sp.	20	10	175
Trichodorus sp.	0	8	125
P. zeae	0	110	35
Dowfume W-85			
Tylenchorhynchus sp.	510	16	25
Trichodorus sp.	0	0	175
P. zeae	16	200	75

[1] "Dowfume MC-2" (98% methyl bromide and 2% chloropicrin) and "Dowfume W-85" (83% ethylene dibromide and 17% naphtha thinner) were supplied by the Dow Chemical Company of Midland, Michigan. "D-D mixture" (1 part dichloropropane and 1 part dichloropropene) was supplied by the Shell Development Company of Denver, Colorado.

Table 2. Yields of corn, from plots given different treatments.

Treatment	Yield in Bushel/Acre
1. Check	68.8
2. Dowfume W-85 @ 7 - 1/2 gal/acre	84.2
3. D-D @ 25 gal/acre	87.7
4. Dowfume MC - 2 @ 2 lbs./100 sq. ft.	94.0
L.S.D. at 5%	9.0
L.S.D. at 1%	12.0

1955, and Dixie 18 corn was planted March 29, 1955, the plants being spaced 15 to 18 inches apart on 42-inch rows. Standard culture practices were followed.

Stand counts and height of plant measurements, taken twice during the growing season, did not reveal any significant differences between treatments and checks. However, examination of the roots showed more extensive root systems in the treated plots. No plant nutritive deficiencies or phytotoxic effects from the fumigants were observed during the growing season. Better than normal growing conditions existed during the early part of the 1955 growing season, but adequate moisture was not available during the silking and tasseling stage of the corn.

One-pint soil samples were taken at random with a standard 12-inch sampling tube from each plot and composited on three different dates during the growing season and the numbers of parasitic nematodes were determined. Table 1 gives a summary of the results.

The plots were harvested September 2, 1955 and the results are given in Table 2. The increases in yields from use of the fumigants were largely due to increases in ear size.

Additional tests will be conducted during the 1956 growing season with the same fumigants and varying rates of nitrogen.

RED RIVER VALLEY EXPERIMENT STATION, LOUISIANA AGRICULTURAL EXPERIMENT STATION, DEPARTMENT OF PLANT PATHOLOGY AND UNITED STATES DEPARTMENT OF AGRICULTURE, AGRICULTURAL RESEARCH SERVICE COOPERATING.

EFFICACY OF SOIL TREATMENTS WITH NINE CHEMICALS IN TOBACCO BLACK SHANK CONTROL

William Lautz [1]

Abstract

Samples of soil infested with Phytophthora parasitica var. nicotianae were each saturated with one of several solutions of eight chemicals and with methyl bromide gas in experiments conducted during a 5-year period. This treated soil, as well as infested untreated soil and uninfested untreated soil, was put into 1-gallon glazed crocks. A tobacco plant of the variety 402 approximately 6 inches high was transplanted into each of the crocks. Methyl bromide, applied at the rate of 1 pound per 100 square feet, controlled black shank. Vapam (Stauffer N-869) (40% sodium N-methyl dithiocarbamate dihydrate) at 1 gallon to 40 gallons, Nabam (Rohm and Haas Dithane D-14) (19% disodium ethylene bisdithiocarbamate) at 1/50, and formaldehyde at 1/25 were effective as drenches for use in the control of black shank. Treatments with some chemicals, including sodium azide and urea, gave control in some years but not in others.

INTRODUCTION

Lucas and Moore (2) in 1952 controlled black shank in plant beds with methyl bromide. Formalin, copper sulfate, and Nabam were used with some success in Kentucky in 1952 (6). Nusbaum and Chaplin in North Carolina reduced the incidence of black shank on Dixie Bright 101 with ethylene dibromide (3). Formaldehyde and Nabam have been used to eradicate black shank where diseased plants were removed from the field (1 and 5). In these tests, tobacco plants were grown in glazed crocks containing black shank soil which had been treated with chemicals.

METHODS AND RESULTS

In July 1952, 45 glazed 1-gallon crocks containing black shank infested soil and 10 crocks of steam sterilized soil were set in a field. Thirty-five crocks of the infested soil were treated in lots of 5 each with 1300 milliliters of seven different chemical solutions. Thirty-three days later these crocks were moved into the greenhouse. One tobacco plant approximately 6 inches high of 402 variety was transplanted in each crock. The rate at which the plants died, up to 54 days after transplanting into treated infested soil, is shown in Table 1.

In October 1952, methyl bromide was applied to 5 crocks of infested soil at the rate of 1 pound per 100 square feet for a period of 48 hours. Five solutions containing urea or sodium azide were each applied to lots of five crocks. One week later a 6-inch tobacco plant was transplanted into each crock. All plants were alive 3 months after planting with the exception of the plants in the untreated infested soil. The roots were examined to determine infection. Table 2 shows disease indices as well as chemical injury to the plants.

In July 1953, 6 compounds were applied to each of 6 lots of 5 crocks, set in a field. One hundred and two days later a tobacco plant was transplanted into each crock in the greenhouse. The number of plants dead, 56 days after planting, in shown in Table 3.

In February 1954, 10 1-gallon samples of black shank infested soil were each treated with one of eight different chemical solutions and with water. The treated samples were allowed to stand for 40 hours, mixed with 3 gallons of sterile soil, and each treatment divided among 5 crocks. A tobacco plant was set in each crock. The rate at which the plants died, up to 35 days after setting in the plants, is shown in Table 4.

In February 1955, 10 1 1/2-gallon samples of black shank infested soil in 3-gallon pails were each treated with 2500 milliliters of solutions of 6 different chemicals, and with water. Thirteen days later, each sample was mixed with 2 1/2 gallons of sterile soil in 8-gallon pails and divided among 5 crocks. One week later the plants were set in the crocks. All the plants were alive 50 days after planting. Disease indices are shown in Table 5.

[1] Plant Pathologist, Field Crops Research Branch, Agricultural Research Service, United States Department of Agriculture.

TABLE I

EFFECT OF CHEMICAL TREATMENT OF SOIL ON BLACK SHANK INFECTION - 1952

Treatment and number of plants		Progress of disease
STERILE SOIL (10)		none dead
UNTREATED BLACK SHANK INFESTED SOIL (10)		1 3 5 7 8 9 10 Plants dead* No.
INFESTED - TREATED WITH CHLOROBROMOPROPENE 3/100	(5)	1 3 4
FORMALDEHYDE 1/25	(5)	1 2 4 5
FORMALDEHYDE 1/100	(5)	2 4 5
SODIUM AZIDE 6 POUNDS PER 100 GALLONS	(5)	1
DITHANE D-14 1/100	(5)	2 5
DITHANE D-14 1/400	(5)	3 5
COPPER SULFATE 1 POUND PER 100 GALLONS	(5)	1 2 3 5
NUMBER OF DAYS AFTER SETTING PLANTS IN CROCKS		10 14 28 31 35 41 46 49 54

Table 2. Effect of soil treatments on incidence of black shank on roots of tobacco. 1952-53.

Treatment	Nov. 3, 1952 Plants set	Plants examined February 9, 1953. Black shank infected		
	No.	No.	Disease index of infected plants	Chemical Injury
Untreated infested soil	10	10	4	-
Healthy soil - check	10	1	1	-
Methyl bromide, 1 lb/100 sq. ft.	5	1	1/8	none
Urea, 100 lbs/100 gallons	5	0	-	very severe
Urea, 50 lbs/100 gallons	5	0	-	severe
Urea, 50 lbs/100 gals. + allyl bromide, 6 qts/100 gals.	5	1	3	slight
Urea, 50 lbs/100 gals. + chlorobromopropene 55, 3 gals/100 gals.	5	0	-	moderate
Urea, 50 lbs/100 gals. + sodium azide, 6 lbs/100 gals.	5	0	-	severe
Sodium azide, 6 lbs/100 gals.	5	0	-	severe
Allyl bromide, 6 qts/100 gals.	5	0	-	slight

Table 3. Effect of chemical treatment of soil on black shank infection. 1953.

Treatment	Original Number of Plants	Number Surviving after 56 days
Sterile soil	10	9
Untreated Black shank infested soil	10	0
Infested soil - treated with formaldehyde 1/25	5	5
Dithane D-14 1/100	5	4
Urea - 50 pounds per 100 gallons	5	5
Urea - 10 pounds per 100 gallons	5	5
Sodium azide - 6 pounds per 100 gallons	5	5
Allyl bromide - 6 quarts per 100 gallons	5	4

TABLE 4

EFFECT OF CHEMICAL TREATMENT OF SOIL ON BLACK SHANK INFECTION – 1954.

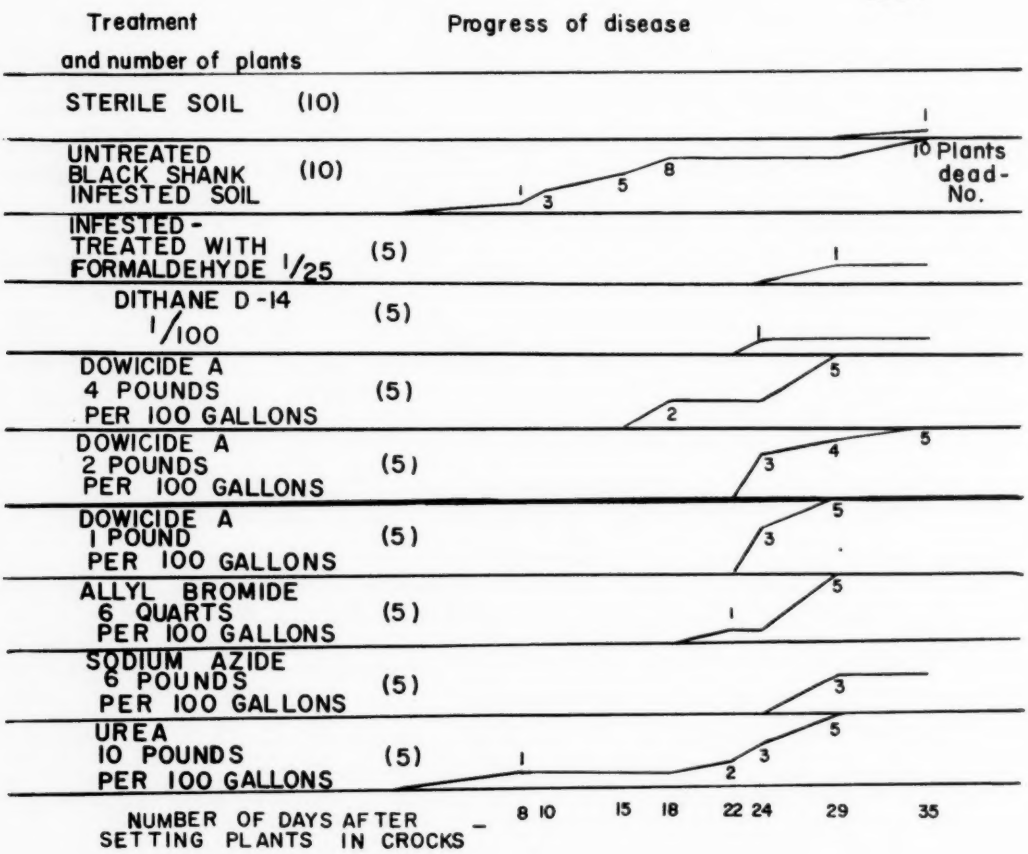

Treatment and number of plants
Progress of disease
STERILE SOIL (10)
UNTREATED BLACK SHANK INFESTED SOIL (10)
INFESTED - TREATED WITH FORMALDEHYDE 1/25 (5)
DITHANE D-14 1/100 (5)
DOWICIDE A 4 POUNDS PER 100 GALLONS (5)
DOWICIDE A 2 POUNDS PER 100 GALLONS (5)
DOWICIDE A 1 POUND PER 100 GALLONS (5)
ALLYL BROMIDE 6 QUARTS PER 100 GALLONS (5)
SODIUM AZIDE 6 POUNDS PER 100 GALLONS (5)
UREA 10 POUNDS PER 100 GALLONS (5)
10 Plants dead-No.
NUMBER OF DAYS AFTER SETTING PLANTS IN CROCKS –
8 10 15 18 22 24 29 35

Table 5. Effect of chemical treatment of soil on black shank infection. Spring 1955.

Treatment	Feb. 24, 1955 Plants set	Plants examined April 15, 1955		
		Black shank infected		
	No.	No.	Mean disease index	Mean plant height inches
Healthy soil	10	0	0	40
Untreated infested soil	10	10	4	17
Chlorobromopropene 3/100	5	5	2.6	26
Dithane D-14 1/100 (Disodium ethylene bis-dithiocarbamate)	5	5	0.7	41
Dithane D-14 1/50	5	5	0.1	39
Formaldehyde 1/25	5	5	0.1	40
Sodium azide 6 lbs./100 gal.	5	5	1.8	26
Urea 50 lbs./100 gal.	5	5	3.8	24
Urea 100 lbs./100 gal.	5	5	4	31
N-869 1 qt/15 gal. (40% N-methyl dithiocarbamate dihydrate)	5	5	1.4	31

Table 6. Effect of chemical treatment of soil on black shank infection. Fall 1955.

Treatment	Nov. 6, 1955 Plants set	Plants examined December 20, 1955			
		Condition of Plants			
		Black shank infected		Black root rot infected	
	No.	No.	Disease index of Infected plants	No.	Disease index of Infected plants
Healthy soil	10	0	-	0	-
Untreated infested soil	10	10	dead	-	-
N-869 1/40 (40% N-methyl dithiocarbamate dihydrate)	10	0	-	0	-
Dithane D-14 1/100 (Disodium ethylene bisdithiocarbamate)	10	0	-	10	2
Dithane D-14 1/50	10	0	-	5	1
Formaldehyde 1/25 (40% commercial formaldehyde)	10	0	-	2	Tr.-slight

In September 1955, 5-gallon samples of black shank infested soil were treated in 10-gallon pails each with one five quart drench of four different chemical solutions and with water. After 24 hours 2 gallons of sterile soil were added to each 5 gallons. The soil was divided among 10 crocks. Since the first plants inserted showed chemical injury, they were removed and replaced with new plants 48 days after the first planting. Fourteen days after planting, all tobacco plants in infested untreated soil were dead. Forty-four days after planting all surviving plants had reached maturity and were examined; disease indices are given in Table 6. Black root rot indices are given because the black shank infested soil was contaminated with black root rot (Thielaviopsis basicola).

DISCUSSION

The most effective chemicals used in these experimental plantings for controlling black shank in infested soils were methyl bromide, Dithane D-14, formaldehyde, and Stauffer N-869. Urea, sodium azide and allyl bromide controlled black shank in one test, but not completely in others. They also produced chemical injury under some conditions. Chemical injury was reduced by allowing the treated soil to stand for two to three months.

Results of greenhouse tests of this type can be used in planning treatments of plant beds and small plots, as well as infested portions of lightly contaminated fields. No method of controlling black shank with chemical drenches in large areas has been found.

Literature Cited

1. Heggestad, H. E., J. O. Andes, and J. Hugh Felts. 1951. Black shank of tobacco. University of Tennessee Extension Service Leaflet 122.
2. Lucas, G. B., and E. L. Moore. 1952. Black shank control in tobacco plant beds. (Abs.) Phytopathology 42: 285-286.
3. Nusbaum, C. J., and J. F. Chaplin. 1952. Reduction of the incidence of black shank in resistant tobacco varieties by soil fumigation. Phytopathology 42: 15.
4. Steinberg, Robert A., and E. E. Clayton. 1948. Chemical soil treatment for black root rot of tobacco in the greenhouse. Phytopathology 39: 155-157.
5. Valleau, W. D. 1951. A program for control of black shank based on a new conception of the life history of the fungus. Bulletin Kentucky Agr. Station 576.
6. Valleau, W. D., E. M. Johnson, and J. W. Irvine. 1952. Experiences with control of black shank of tobacco in 1952. Bulletin Kentucky Agr. Station 592.

FIELD CROPS RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE,
UNITED STATES DEPARTMENT OF AGRICULTURE

CHEMICALS SCREENED FOR SYSTEMIC EFFECTS AGAINST SPREADING DECLINE DISEASE OF CITRUS [1]

Harry W. Ford

Abstract

A total of 133 chemicals were screened for systemic effects against spreading decline. At the present time no material has shown promise based on stimulation of new shoot growth by diseased trees.

Present control measures recommended for spreading decline caused by the burrowing nematode, Radopholus similis (Cobb) Thorne, consist of removing trees and fumigating the soil with D-D (2). This is an expensive procedure with several years elapsing before replanted citrus trees are again in production. An effective means of combatting the nematode attack without destroying the trees would be of immeasureable value in the spreading decline control program. One phase of the research underway is the search for a systemic material that will revitalize decline trees and prevent the nematodes from feeding in roots. The approach to the program initiated in 1954 consisted of injecting chemicals into diseased trees in commercial groves. It was thus possible to observe toxic or beneficial effects on tree growth. It was also considered a remote possibility that a compound showing no toxicity to nematodes in vitro might act as a nematode repellant within the plant.

In the absence of information on the downward translocation of chemicals in the plant and only meager knowledge of their physiological or nematocidal properties, the test treatments represented a wide variety of substances. Preliminnary screening was based on stimulation of tree growth, an essential criterion if a treatment was to be of economic value. Any treatment that stimulated tree growth in the screening tests was to be studied in greater detail.

METHODS

In the procedure used, the test materials were first mixed in a carbowax carrier (polyethylene glycol 1500). When necessary a small quantity of water was added to the carbowax so that the test material could be easily incorporated. The mixture was placed in a 1 pint polyethylene bag and transported to the grove. Eight to 12 one-inch diameter holes were drilled into the trunk of the tree to a depth of 1.5 inches. The mouth of the plastic bag was clamped to the base of a 5-inch by 7/8-inch stainless steel tube. The tube was inserted to the bottom of the hole and slowly removed as the test material was injected by squeezing the bag. The holes were covered with masking tape and sealed with asphalt pruning paint. Three weeks were usually required for total absorption, at which time the holes were swabbed with an antiseptic pruning paint.

Trees from four groves were used in these studies. One block of 30-year-old Valencia oranges had shown symptoms of spreading decline for at least four years. The trees were injected in 1954 using one tree for each chemical. The second grove consisted of 10-year-old Hamlin oranges recently invaded by the burrowing nematode. The 13-year-old trees in the third grove were replants in decline infested soil following removal of older diseased trees. Trees in the fourth grove were 30-year-old orange trees infested with the burrowing nematode for about 5 years. Sufficient trees were available in the last three groves so that two trees were injected with each test material in 1955.

---

[1] Florida Agricultural Experiment Station Journal Series, No. 517.

Table 1. Materials affecting tree growth.

Material	Amount	Age of tree Years	Rating after injection[a] 3 mo.	6 mo.	1 yr.
Anthelmintics					
Substituted acetylene 54R5911 (Merck)	3g	30	1	3	3
Antibiotics					
Rumocidin (Pfizer)	8g	30	3	1	1
Thiolutin (Pfizer)	9g	30	2	1	1
Organic compounds					
Benzyl parahydroxybenzoic acid	15g	30	2	1	1
Beta-carboxyethyl benzothiazyl-2-sulfide	20g	30	3	1	1
2,6 dihydroxy 4 carboxypyridine	25g	30	1	3	2
2,6 dihydroxy 4 carboxypyridine	10g	10	2	1	1
2,6 dihydroxy 4 carboxypyridine	15g	13	0	0	1
Octyl phenol	15g	13	0	0	0
Pentachloroethane	15g	13	0	1	1
Propargyl bromide	7ml	13	0	dead	
Systemic insecticides					
Thimet	25ml	13	1	1	2
Systox	17ml	13	0	1	2

[a] Rating value
0 - visible damage to the tree.
1 - No effect in comparison to untreated decline trees.
2 - Improved short growth of small leaves frequently on only several branches.
3 - Improved growth with normal size leaves frequently on only one half of tree.
4 - Pronounced and uniform stimulation of growth.
5 - Growth comparable to a healthy tree.

RESULTS

A list of material screened in 1954 and 1955 that affected tree growth is shown in Table 1. A rating of "0" indicated that the chemical was toxic to the tree. A rating of "1" signified no tree response in comparison to untreated decline trees while a rate of "5" signified growth comparable to a healthy tree. Materials that had no effect on tree growth are shown in Table 2.

At the present time no material has shown promise as a systemic toxicant for killing or repelling the burrowing nematode based on stimulation of new shoot growth by diseased trees. Substituted acetylene stimulated tree growth in the first grove and was repeated in the other three groves with insignificant results.

The data do show that citrus may differ from other plants in tolerance to certain compounds For example, 30 grams of alpha-methoxylphenylacetic acid did not injure a mature citrus tree but low concentrations are known to be detrimental to tomatoes (1). In contrast, Citrazinic

Table 2. Materials not affecting tree growth.[a]

MATERIAL	AMOUNT
Anthelmintics	
Amino acid derivative (Merck)	2.5g.
Methyl sulfate derivative (Merck)	1.5g.
Subst. acetylene 55R1577 (Merck)	7.5g.
Subst. acetylene 55R1578 (Merck)	7.5g.
Subst. acetylene 54R5911 (Merck)	15g.
Antibiotics	
Bacitracin	5g.
Magnamycin	5g.
Polymyxin B Sulfate	5g.
Potassium penicillin	5g.
Streptomycin sulfate	5g.
Terramycin HCl	5g.
Fungicides	
Copper 8 hydroxyquinoline	10g.
Cuprous oxide	20g.
2,3-dichloro-1,4-napthoquinone	20g.
Diethyldithiocarbamic phosphoro-dithioic anhydrosulfide O, O-diisopropyl ester	30g.
3,5 dimethyl-tetrahydro-1,3,5, 2H-thiodiazine-2-thione	15g.
8 hydroxyl quinoline sulfate	15g.
Maneb	15g.
3 para-chlorophenyl-5-methyl-rhodamine	15g.
Sodium Chloro-2-phenylphenate	25g.
Sodium orthophenylphenate	25g.
Tetramethyl thiuram disulfide	25g.
Zineb	20g.
Insecticides	
Aldrin	35g.
Aramite	30g.
Chlordane	25g.
Chlorinate camphene	10g.
Chlorobenzilate	35g.
Ethyl p-nitrophenyl thionobenzene-phosphonate	20g.
Malathion	20g.
Mitox	20g.
Ovotran	20g.
Parathion	15g.
Phenyl mercuri triethanol ammonium-lactate	15g.
Tetraethyl pyrophosphate	25ml.
1,1,1-trichloro-2, 2-bis (p-chlo-rophenyl)-ethane	20g.
Nematocide	
1,2-dibromo-3-chloropropane	10ml.
O-2,4 dichlorophenyl O, O-diethyl-phosphorothionate	15ml.

Table 2. (Continued)

MATERIAL	AMOUNT
Organic compounds	
Benzophenone	15g
Butylacrylamide	20g.
Calcium resinoleate	10g.
Chloroacetaldehyde	17ml.
Chlorocyclohexene	20ml.
Cochineal powder	20g.
Copper phenylsalicylate	20g.
Croton aldehyde	20ml.
Cyclohexanol	25ml.
Cyclo hexanone	25 ml.
Decalin	30ml.
Diallylmelamine	30g.
Dibromodimethylhydantoin	15g.
Dibromo-8-hydroxyquinoline	20g.
4,4 dichlorodiphenyl	10g.
Dichloromethane	20g.
Dichloronaphthalene	10g.
2,4 dichlorophenol	25g.
Diethyl-maleate	20ml
Dimethyl hexynediol	30g.
Dimethyl octynediol	35g.
Dimethyl phthalate	40ml
4,6 dinitro-O-cresol	30g.
2,4 dinitrophenol	20g.
Dipentene	30ml
Diphenyl	20g.
Diphenyl-thio-carbazone	30g.
Glycerol mono oleate	25ml
Methyl parahydroxybenzoic acid	15g.
Nitrofurfuryl beta chloropropionate	10g.
5 nitrofurfuryl bromoacetate	5g.
5 nitrofurfuryl-p-chlorobenzoate	5g.
Nonyl phenol	25ml
Octyl resorsinol	15g.
Ortho chlorophenol	10ml
Para chlorophenol	30g.
Paraformaldehyde	10g.
Paramethyl aminophenol	25g.
Potassium chrome alum	20g.
Propylene glycol	25ml.
Propyl parahydroxybenzoic acid	15g.
Pyrogallic acid	20g.
Terpene alcohol	25 ml
Thiourea	15g.
Vanillin	25g.
Sulfonomides	
Sulfamethazine	2.5g.
Sulfanilamide	12.5g
Sulfanilamide derivative	2.5g.
Sulfapyridine	15g.
Sulfaquinoxaline	15g.
Sulfasuxidine	10g.
Sulfathalidine	10g.

(Table 2. (Concluded).

MATERIAL	AMOUNT
Systemic insecticides	
0,0 diethyl O-(3 methyl pyrazolyl-(5)-dimethylcarbamate	25ml.
0,0 diethyl S-isopropyl mercaptomethyl-dithiophosphate	25ml
0,0 diethyl S-n-propylmercaptomethyl-dithiophosphate	25ml
0,0 dimethyl O-2 ethylmercaptoethyl thiophosphate	25ml
1-isopropyl-3 methyl-pyrazolyl-(5)-dimethylcarbamate	25ml

[a] In addition, 26 materials were tested under company code designation for which chemical names were unavailable.

acid (2, 6 dihydroxy 4 carboxy pyridine) stimulated the growth of a mature orange tree but damaged a 13-year-old tree. The slow rate of absorption may have been a factor in the low incidence of tree damage by the test treatments. In addition, certain of the materials may have moved only a short distance in the trunk.

Literature Cited

1. Mitchell, John W., and W. H. Preston. 1953. Secondary galls and other plant growth modifying effects induced by translocated alpha methoxylphenylactic acid. Science 118: 518-519.
2. Suit, R. F. 1954. The experimental basis and limitations of the pull and treat method of handling spreading decline. Proc. Florida State Hort. Soc. 67: 85-89.

FLORIDA CITRUS EXPERIMENT STATION, LAKE ALFRED

NEW AND SUSPECTED HOST PLANTS OF THE BURROWING NEMATODE, RADOPHOLUS SIMILIS (COBB) THORNE

Wray Birchfield

Abstract

Thirty-one plants are reported as new hosts of the burrowing nematode on the basis of finding all stages of the organism in the root tissues. Twenty-four additional plants are indicated as suspected hosts on the basis of finding considerable numbers of the organism in different stages in the drainage water of incubated plant roots. These hosts and suspected hosts represent 38 plant families.

INTRODUCTION

Since the original work of Cobb (3) who reported the burrowing nematode in lesions on banana and sugarcane roots, other host plants were compiled and reported by Filipjev and Schuurmans Stekhoven (4), and Goodey (5). More recently Brooks (1, 2) reported many new hosts of this organism from Florida while Sher (6) listed a few from Hawaii.

The State Plant Board of Florida has maintained a complete list of host and suspected host plants for the benefit of survey teams working on spreading decline of citrus and also for nursery inspectors as a guide in their sampling operations. Continual efforts are being made to keep this list as completely up to date as possible.

Acknowledgments are made to the services rendered by the personnel of the U. S. Department of Agriculture Plant Pest Control, the University of Florida Citrus Experiment Station and the State Plant Board of Florida, who collected and examined by far the majority of the new host plants herein reported. The necessity of such studies and the importance of a host list as complete as possible, is obvious.

MATERIALS AND METHODS

Root samples of a large variety of plants associated with infected citrus groves were collected. Additional samples were taken from ornamental plants and miscellaneous nursery plantings. Several plants were inoculated with the burrowing nematode under controlled conditions in the slathouse and in temperature tanks.

The root samples were processed by the method reported by Young (7). That is, about one-half pint of roots from a given plant were thoroughly washed and stored in a moist condition in mason jars. After about 3 to 5 days these roots were examined for the burrowing nematode by either of two methods: 1) the roots were atomized and the drainage water examined, or 2) water was added to the roots and passed through a 325 mesh screen which retained the nematodes. When nematodes in sufficient numbers were recovered by this method, the plant was placed on the suspected host list. Care was taken at the time of sampling to obtain only the roots from the plant in question. Roots from the suspected host plant were then dissected to obtain the burrowing nematode in all stages of development. If the nematodes were found under this condition it was assumed that they were successfully reproducing and therefore, the plant was listed as a host.

RESULTS

Thirty-one plants were listed as new hosts of the burrowing nematode on the basis of finding all stages of the organism in the root tissues. Twenty-four additional plants are suggested as suspected host plants on the basis of finding considerable numbers in the drainage from incubated roots. The scientific and common name and the plant family of these hosts and suspected hosts are listed in Tables 1 and 2, respectively.

Table 1. New host plants of the burrowing nematode, Radopholus similis (Cobb) Thorne.

Scientific Name	Common Name	Plant Family
Buxus microphylla var. japonica Rehd. and Wilson	Japanese boxwood	Buxaceae
Calathea lietzei Morr.	Calathea	Marantaceae
Calathea ornata Koern.	Calathea	Marantaceae
Diospyros kaki L.	Japanese persimmon	Ebenaceae
Eriobotrya japonica Lindl.	Loquat	Rosaceae
Hibiscus rosa-sinensis L.	Hibiscus	Malvaceae
Indigofera hirsuta L.	Hairy indigo	Leguminosae
Jasminum dichotomum Vahl.	Gold Coast jasmine	Oleaceae
Malpighia glabra L.	Barbados cherry	Malpighiaceae
Musa nana Lour.	Cavendish banana	Musaceae
Pandanus veitchii Dall.	Pandanus	Pandanaceae
Panicum hemitomon Schult.	Maiden cane	Gramineae
Panicum maximum Jacq.	Guinea grass	Gramineae
Peperomia obtusifolia A. Dietr.	Peperomia	Piperaceae
*Philodendron dubium Chod. & Vischer	Philodendron	Araceae
*P. hastatum C. Koch & Sello	Philodendron	Araceae
*P. panduraeforme Kunth	Philodendron	Araceae
*P. selloum C. Koch	Philodendron	Araceae
*P. wendlandii (hybrid)?	Philodendron	Araceae
*P. wendlandii Schott	Philodendron	Araceae
Phoenix canariensis Chabaud.	Canary Is. date palm	Palmaceae
Pilea involucrata Urban	Aluminum plant	Urticaceae
Podocarpus macrophylla Don. var. maki Endl.	Podocarpus	Podocarpaceae
Psidium cattleianum Sabine	Cattley guava	Myrtaceae
Rhynchelytrum roseum (Nees) Stapf & Hubb.	Natal grass	Gramineae
Ricinus communis L.	Castor bean	Euphorbiaceae
Ruellia makoyana Hort.	Ruellia	Acanthaceae
Smilax lanceolata L.	Jacksonbrier	Smilacaceae
Vinca rosea L.	Periwinkle	Apocynaceae

* Validity of name not sufficiently investigated.

DISCUSSION

There may be some doubt as to the value of listing a plant as a suspected nematode host. The chances of a root sample being mixed are many; however, where the endoparasitic, burrowing nematode was found in substantial numbers in the drainage water of washed incubated roots, very little difficulty was generally experienced in dissecting the organism from the tissues. It is expected that most of the plants listed as suspected hosts will eventually be placed on the new host list as the work involving dissection progresses.

It is conceivable that nematodes may reproduce on a given plant to a limited scale and ultimately fail to survive.

Table 2. Suspected host plants of the burrowing nematode Radopholus similis (Cobb) Thorne.

Scientific Name	Common Name	Plant Family
Albizzia lebbek Benth.	Woman's tongue	Leguminosae
Alpinia speciosa Schum.	Shell lily	Zingiberaceae
Anthurium crystallinum Lind. & André.	Anthurium	Araceae
*Anthurium wrightii?	Anthurium	Araceae
Beloperone guttata Brandeg.	Shrimp plant	Acanthaceae
Cinnamomum camphora Nees. & Eberm.	Camphor tree	Lauraceae
Collinia elegans Liebm.	Parlor palm	Palmaceae
Delonix regia Raf.	Royal poinciana	Leguminosae
Feijoa sellowiana Berg.	Feijoa	Myrtaceae
Gardenia jasminoides Ellis	Cape jasmine	Rubiaceae
Jasminum bahiense DC.	Jasmine	Oleaceae
Jasminum amplexicaule Don	Jasmine	Oleaceae
Magnolia grandiflora L.	Magnolia	Magnoliaceae
Maranta leuconeura var. kerchoveana Morr.	Maranta	Marantaceae
*Philodendron cordatum Kunth	Philodendron	Araceae
P. imbe Schott.	Philodendron	Araceae
*P. mandaianum?	Philodendron	Araceae
*P. melinonii Brongn, ex Regel	Philodendron	Araceae
*P. micans C. Koch	Philodendron	Araceae
*P. pitheri ?	Philodendron	Araceae
*P. rubrum ?	Philodendron	Araceae
*P. sagitatum	Philodendron	Araceae
P. sodiroi Hort.	Philodendron	Araceae
P. wendlandii X imbe	Philodendron	Araceae

*Validity of name not sufficiently investigated.

Literature Cited

1. Brooks, Troy L. 1955. Additional hosts of the burrowing nematode. Plant Disease Reporter. 39: 309.
2. _____. 1954. The host range of the burrowing nematode internationally and in Florida. Proc. Fla. Hort. Soc. 67: 81-83.
3. Cobb, N. A. 1915. Tylenchulus similis, the cause of a root disease of sugarcane and banana. Jour. Agr. Res. IV: 561-568.
4. Filipjev, I. N. and J. J. Schuurmans Stekhoven, Jr. 1941. A manual of Agricultural Helminthology. E. J. Brill. Leiden, Holland. 878 pp.
5. Goodey, T. 1951. Soil and freshwater nematodes. John Wiley and Sons, Inc. N. Y. 390 pp.
6. Sher, S. A. 1954. Observations on plant parasitic nematodes in Hawaii. Plant Disease Reporter. 38: 687-689.
7. Young, T. W. 1954. An incubation method for collecting migratory endoparasitic nematodes. Plant Disease Reporter. 38: 794-795.

STATE PLANT BOARD OF FLORIDA, GAINESVILLE

OCCURRENCE OF CYST NEMATODES, HETERODERA SPP., IN MARYLAND[1]

W. R. Jenkins, B. W. Coursen, R. A. Rohde, and D. P. Taylor

During a nematode survey of the northern counties of the State of Maryland, cysts of the nematode genus Heterodera were found in 77 of 260 farms sampled, an incidence of 29.6 percent. All cysts recovered in these counties were of the Heterodera schachtii group and wherever second stage larvae were available for species identification, the form was found to be Heterodera trifolii (Goffart, 1932) Oostenbrink, 1949, the clover-cyst nematode.

Because clover is used in rotation in nearly all the fields sampled and occurs throughout the area in lawns, along roadsides, edges of fields, and persists as volunteer plants in many fields, it is thought likely that most of these cysts were of H. trifolii.

The importance to the growth of clover in these counties cannot be determined at this time, although this nematode is known to be a pathogen capable of causing severe losses in clover [2]. This crop in Maryland is frequently very poor and in many cases poor production has been associated with the presence of this nematode.

One sample from Talbot County and two samples from Caroline County were collected from soybean fields and contained cysts of the H. schachtii group. No larvae were recovered and cysts contained no embryonated eggs; therefore, no species identification was possible.

A second species of cyst nematode has also been recorded from Maryland. Cysts identified as members of the H. cacti group were recovered from one field in Wicomico County and one field in Worcester County on the Eastern Shore. There was no evidence that this nematode was parasitizing soybeans although no other plant was present in the area sampled.

Counties in which Heterodera spp. have been discovered are shown in Figure 1.

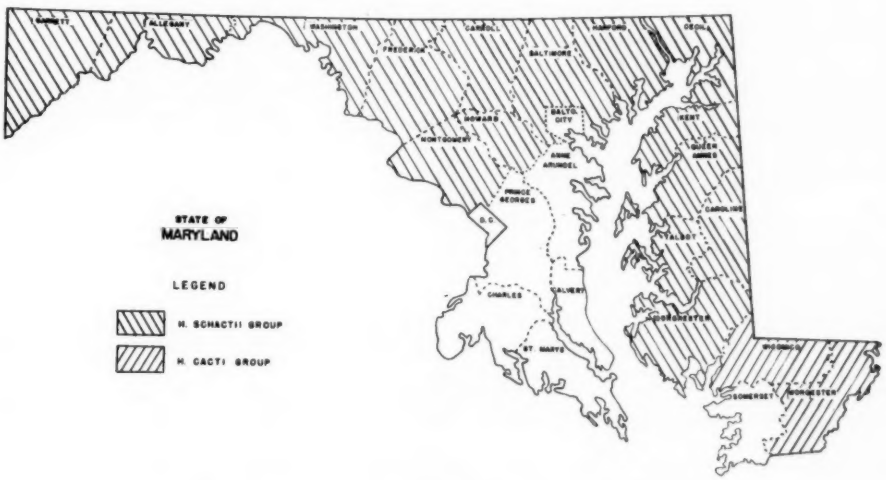


FIGURE 1. Occurrence by county of Heterodera spp. in Maryland.

DEPARTMENT OF BOTANY, UNIVERSITY OF MARYLAND, COLLEGE PARK, MARYLAND

[1] Scientific Article No. A581, Contribution No. 2744 of the University of Maryland Agricultural Experiment Station, Department of Botany.

[2] Gerdemann, J. W. and M. B. Linford. 1953. A cyst-forming nematode attacking clovers in Illinois. Phytopathology 43: 603-608.

ERADICATION OF PECAN SCAB ON NURSERY TREES

Richard H. Converse [1]

Cladosporium effusum (Wint.) Demaree, the incitant of pecan scab, persists on the tree from one growing season to the next as holdover stromata on infected parts. On nursery trees lesions may occur on the trunk and main branches as well as on the smaller branches. Scab-infected nursery stock can become a source of inoculum in old or new plantings. The shipment of infected nursery stock could be an important means of distributing possible new strains of the scab pathogen. For example, a strain of C. effusum capable of infecting the variety Stuart, long regarded as highly resistant to scab, was recently reported in Mississippi [2].

In March, 1956, 4 materials found promising as contact fungicides [3] when tested against the pecan scab pathogen in the laboratory and the field [4] were sprayed on badly scabbed nursery

Table 1. Effects of application of contact fungicides to dormant nursery trees of Burkett pecan on sporulation of holdover stromata of the scab fungus on stems and on foliation, Stillwater, Oklahoma, 1956.

Contact fungicides and number of applications	Amount of fungicide in 100 gal. of diluted spray material	Trees treated	Average sporulation rating[a] of holdover stromata after indicated period		Nursery trees that foliated
		No.	10 days	112 days	Percent
Puratized Agricultural Spray					
2 applications	5 pints	5	0	0	100
1 application	5 pints	7	0	0	100
1 application	2.5 pints	7	0	0.3	100
Santobrite + Standard Dormant Spray Oil No. 1 1 application	4.7 pounds + 1 gallon	7	0.1	0.1	100
Stauffer N-521 1 application	2.8 pounds	7	3.7	1.0	86
Dithane D-14 1 application	1.9 pints	7	1.1	0.6	100
None (Check)	____	4	4.0	2.3	100

[a] Based on a 0-4 scale: 0 = no conidia; 4 = heavy sporulation.

1 Assistant Plant Pathologist and Agent, United States Department of Agriculture.

2 Cole, J. R. and A. C. Gossard. 1956. Stuart pecan found to be susceptible to scab in Mississippi. U. S. Dept. Agr., Plant Dis. Reptr. 40: 156.

3 The phrase "contact fungicides" is used in the sense of J. G. Horsfall (1956, "Principles of Fungicidal Action" Waltham, Chronica Botanica Co.) to include materials that destroy the entire thalli of fungi exposed to the materials. The term replaces "eradicant fungicides", previously used to denote this group of chemicals.

4 Converse, R. H. 1956. Further evidence of the value of eradicant fungicides for control of pecan scab. (Abs.) Phytopathology 46: 9.

trees of the variety Burkett, which had just been planted. A knapsack-type sprayer was used to apply them. The 4 compounds used as fungicidal treatments in this experiment were:

Puratized Agricultural Spray -- 7.5% tris (2-hydroxyethyl) (phenylmercuri) ammonium lactate
Santobrite -- 75% sodium pentachlorophenoxide + 13% sodium salts of other chlorophenols
Stauffer N-521 -- 90% tetrahydro — 3,5-dimethyl-2H-1,3,5-thiadiazine-2-thione
Dithane D-14 -- 19% nabam (disodium ethylenebis (dithiocarbamate))

Single applications were made of each of the 4 materials. In addition, 2 applications of Puratized Agricultural Spray were made to one group of trees, and another group was left unsprayed.

The production of conidia of C. effusum on holdover stromata on stems was determined 10 and 112 days after the contact fungicides were applied. Puratized Agricultural Spray at the 5-pint rate completely prevented the production of conidia (Table 1). Aerial mycelium was present on the stromata after one but not after two applications of Puratized Agricultural Spray. A fair degree of control was obtained with Puratized Agricultural Spray at the 2.5-pint rate and with Santobrite plus dormant oil. Complete eradication of the pathogen is desirable, however, to prevent the development of a few primary lesions on foliage which could serve as sources of conidia for secondary infections. Stauffer N-521 and Dithane D-14 were unsatisfactory in this test.

None of the treatments except Stauffer N-521 on one tree affected foliation (Table 1). The rate of leaf appearance was independent of treatment.

From this test it appears that complete suppression of sporulation on holdover stromata on dormant pecan nursery trees is possible, provided the proper concentration of a satisfactory contact fungicide is used and good coverage is obtained.

OKLAHOMA AGRICULTURAL EXPERIMENT STATION AND HORTICULTURAL CROPS RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, STILLWATER, OKLAHOMA

CONTROL OF BULL'S-EYE ROT ON APPLE AND PEAR FRUITS

J. R. Kienholz [1]

ABSTRACT

Bull's-eye rot, caused by either Neofabraea malicorticis or N. perennans, often causes serious losses on apple and pear fruits in cold storage The influence that winter injury to apple trees and periods of rainfall play in fruit infection cycles in the Pacific Northwest in shown. The discovery that latent infections may develop in young fruits explains why the sprays used prior to 1950 sometimes failed to control the fruit rot. A more effective program for fruit rot control is suggested as a result of recent spray tests.

INTRODUCTION

Several different fungi may cause a bull's-eye type of rot (5) on apple and pear fruits in the Pacific Northwest, but generally the Gloeosporium stage of either the northwestern apple anthracnose fungus (Neofabraea malicorticis Jacks.) or the perennial canker fungus (N. perennans Kienh.) is responsible. These two native fungi (2, 13), and the diseases they cause on orchard trees, were previously compared (6).

Prior to 1950, control of bull's-eye rot by means of orchard sprays was erratic. Good control was obtained by spraying the fruit with a copper fungicide some years, and none in others. Serious skin russeting or fruit spotting was liable to occur from the use of any copper fungicide on Anjou pears or Yellow Newtown apples. The present study was initiated to find a more suitable and effective fungicide for use on these spray-sensitive varieties, as well as to determine why the program being used often failed to control the fruit rot. A new concept of infection of pome fruits in the orchard was developed during the study.

IMPORTANCE OF BULL'S-EYE ROT

Losses from bull's-eye rot are often serious. One example of very heavy loss occurs in the records of a commercial packing house at Hood River, Oregon. During 1950 the quality of 57 percent of the Yellow Newtown apple crop was too poor for fresh-fruit shipment. Although other factors were concerned, as many as 75 percent of the fruit in some individual boxes developed bull's-eye rot. Even the more usual repacking of 2 to 9 percent of the crop during years favorable for fruit infections is costly. Holding fruit for late shipments has been hazardous because of the bull's-eye rot problem. The practice of holding fruit loose in cold storage, and packing just before shipment, has partially reduced repacking costs. Fruit predisposed to bull's-eye rot, however, may develop considerable decay during shipment. The loss of reputation for supplying quality fruit is often more serious in such cases than the monetary loss of the fruit itself.

THE FRUIT ROT

Both the anthracnose and the perennial canker fungus may cause bull's-eye rot on fruit in storage. Certain characteristics have been used to determine the fungi involved (3, 8, 13), but comparisons of several strains of each fungus failed to reveal clear-cut differences in fruit rot symptoms. Each fungus is limited to a definite ecological environment (6). The anthracnose fungus attacks trees in the more humid districts, but the infections are readily controlled by copper fungicides applied during October or November. Sources of anthracnose spores can therefore be eliminated. On the other hand, orchard spraying does not prevent infections by the perennial canker fungus, and since its habitat coincides with the important irrigated apple and pear-producing districts, the fruit rot it causes assumes the dominant role in commercial fruit losses. The same description applies regardless of which fungus infects the fruit.

On fruit held in cold storage, bull's eye rot seldom appears before late December. Fruit held later is often "eating ripe" before the rot develops; therefore the name "ripe spot" is sometimes applied (1, 11).

The rot starts as a small circular, light or dark brown, firm lesion. As it enlarges, con-

1 Pathologist, Horticultural Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Hood River, Oregon.

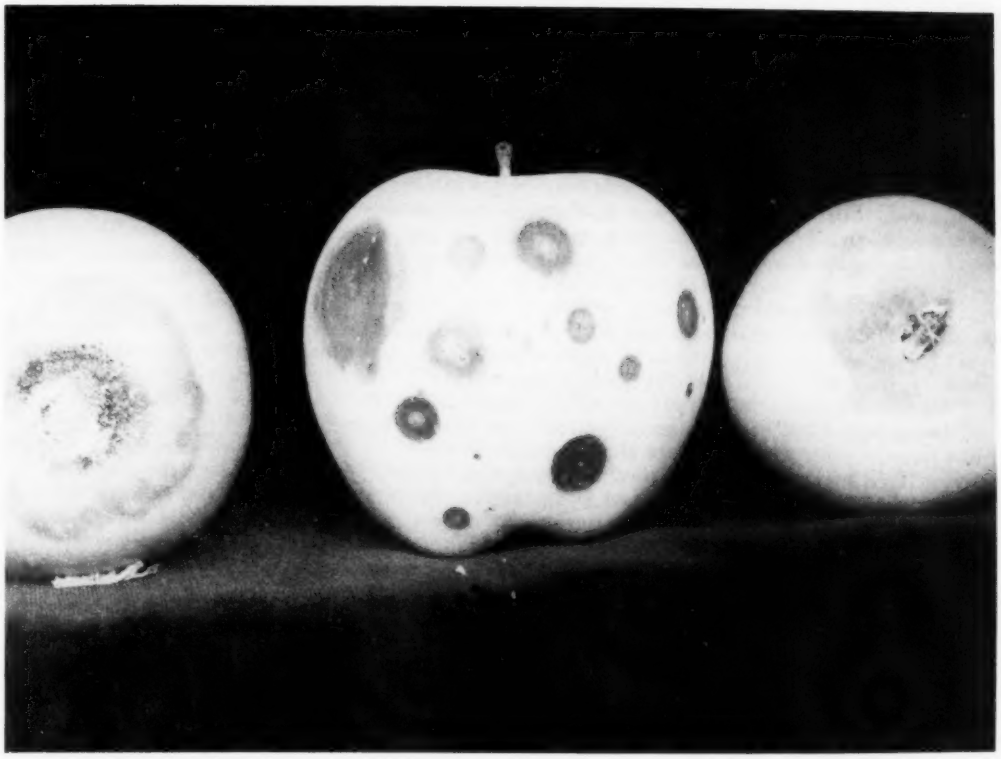


FIGURE 1. Bull's-eye rot on Yellow Newtown apples. Calyx-end rot at left, showing early development of acervuli; the so-called "parasitic" type of lenticel infections at center; stem-end infection at right.

centric zones of lighter and darker tissues sometimes form. This character suggests the name bull's-eye rot. Acervuli break through the skin as the lesion enlarges, mostly in a concentric ring arrangement, and finally a creamy white and slimy mass of conidia may develop on the surface. If the storage atmospheres are too dry only mycelial tufts develop.

Fruit infections may occur at the stem-ends through the pulled stems or punctures, through dead or injured calyx lobes, through other injuries or blemishes, or by way of lenticels (Fig. 1.) Whether the fungus is able to enter through uninjured lenticels or requires microscopic injuries for infection has not been determined. When numerous lenticel infections occur, bull's-eye rot resembles apple bitter rot of the eastern United States (9).

HISTORY OF FRUIT INFECTIONS

Three conditions are necessary for infections of susceptible fruit varieties by the bull's-eye rot fungi : 1) An adequate source of spore material must be presenf in tree cankers; 2) Rainfall must be sufficient to wash the spores onto the fruit; 3) The period of rainfall must persist long enough for the spores to germinate and infect the fruit.

Relation to winter injury: Spores from anthracnose cankers rarely cause serious fruit rot losses unless a proper orchard spray program (12) has been neglected. Spores of the perennial canker fungus from cankers on injured tree bark cause the important fruit rot losses in the irrigated apple and pear districts of eastern Oregon and Washington. The record of one packing plant at Hood River, Oregon (Table 1), shows the general relation between winter-injury periods and cycles of bull's-eye rot infections on Yellow Newtown apples.

Table 1. Relation of repacking of Yellow Newtown apples, largely because of bull's-eye rot in one packing plant at Hood River, Oregon, to occurrence of winter injury and rainfall.

Crop year	Rainfall		Total boxes packed	Crop repacked
	May through August	September and October		
	Inches	Inches	Number	Percent
1932-33 a	1.27	2.42	752,010	0.1
1933-34	2.65	5.12	269,939	6.1
1934-35	.70	5.32	674,868	2.8
1935-36	.54	1.32	506,009	0.2
1936-37 a	4.43	.46	776,385	1.8
1937-38	5.29	1.78	534,281	9.2
1938-39	.45	1.61	807,045	0
1939-40	1.82	.97	490,108	0
1940-41	.71	4.44	770,171	4.9
1941-42	3.44	2.40	392,810	0
1942-43 a	3.13	1.65	370,754	0
1943-44	.92	5.26	407,216	0
1944-45	2.09	2.19	575,259	0
1945-46	3.00	2.08	495,690	0
1946-47	2.96	3.96	544,667	0.2
1947-48	3.54	9.37	565,758	3.3
1948-49	5.28	3.78	499,841	0.4
1949-50 a	2.40	2.98	576,117	0.4
1950-51 a	.91	7.50	377,869	57.0 b
1951-52	1.94	6.17	548,082	3.6
1952-53	2.38	.01	589,483	0
1953-54	2.24	1.03	293,215	0
1954-55	3.52	2.93	617,016	0
1955-56 a	1.59	8.03	480,970	0

a Year when temperatures dropped below zero and winter injury to trees occurred.
b Diverted to cannery use because of breakdown and high rot incidence.

Except for an unexplained increase during 1940, and the relaxation of grading rules during the war from 1941 to 1945, the effect of winter injury on bull's-eye fruit rot increase is apparent. In general, fruit rot increased during the crop years following winter-injury periods and gradually decreased as the trees recovered from the damage.

Relation to early and late rainfall: Table 1 also shows that about 4 inches or more rainfall occurred during September and October in all but 2 of the years when heavy repacking of fruit was necessary. The notable exceptions were in 1936 and 1937, when relatively heavy summer rainfall occurred, indicating that fruit rot infections might have taken place during the summer. The late rainfall of 1936, in particular, was too meager to be of any consequence in causing fruit rot infection. These records, and the erratic control data from early spray tests against the fruit rot, gave an indication that early latent infections on the fruit were a possibility.

Tests for latent infections: Young Yellow Newtown apples, picked during the latter part of May, were placed in moist chambers at room temperature. The perennial canker fungus developed and overran many of the young fruits after a month. The fungus was isolated from these young fruits and subsequently produced typical perennial cankers when used to inoculate trees in the dormant season. It was thus established that fruit infections can occur early in the season. These infections remain latent until the fruit becomes relatively mature. During 1936 and

1937, for example, the majority of infections occurred before September, but the fruit did not rot until after it had been in storage for several months. The demonstration that latent infections can occur therefore forms a logical basis for comparing the effectiveness of early and late spray applications for bull's-eye rot control.

CONTROL TESTS

Disinfectants in the washing tanks: Sodium o-phenylphenate (Stop Mold) used to treat pears in a washing tank during 1947 (7) appeared to offer some promise of control of bull's-eye rot. In a similar test in 1948 the same material had no effect on this rot. It seems probable that disinfectants affect fruit rot fungi only if they are exposed on the fruit surface. Early infections probably become so deep-seated and protected that disinfectants cannot reach them. Commercial use of disinfectants in washing tanks have had little effect on bull's-eye rot.

Effect of fungicides on spore germination: Preliminary tests on the effects of captan, copper oxychloride, dichlone, ferbam, ziram, and Karathane indicated that ziram, dichlone and captan at concentrations of approximately 1 to 100,000 were about equally effective in preventing the germination of Gloeosporium conidia. Ziram caused plasmolysis of many of the spores, whereas the other fungicides did not. It was used exclusively in the 1952 to 1954 spray tests.

Spray tests since 1950: All sprayed plots were set up so that at least 100 to 200 boxes of fruit would be available for sampling. From this field sample 30 to 50 boxes were used at random for the disease counts. Records were taken when the fruit was being shipped from cold storage to the late market. In calculation of the percentage of fruit with rot every affected fruit was included whether it had one infection or many. The results of spray tests on Yellow Newtown apples during a 4-year period are summarized in Table 2.

Table 2. Bull's-eye rot on cold-storage Yellow Newtown apples from trees sprayed with various chemicals, Hood River, Oregon, 1951-54.

Year and spray	Date sprayed	Date fruit examined	Rainfall (inches)		Total number of apples	Percent bull's-eye rot
			May through August	September through October		
1951:						
None					3173	7.9
Ziram 1 1/2-100	7/25 & 9/18	4/1/52	1.94	6.17	3192	1.8
Captan 2-100	Do.				3128	1.8
Copper dithiocarbamate 1 1/2 -100	Do.				2955	1.6
1952:						
None	-				3980	1.5
Ziram 1 1/2-100	5/12 & 5/27	3/12/53	2.38	.01	4019	0.9
Do.	7/24, 8/18, & 9/25				4075	1.0
Do.	5/12, 5/27, 7/24, 8/18 & 9/25				2356	0.8
1953:						
None	-				3722	6.3
Ziram 1 1/2-100	5/18 & 5/28	5/12/54	2.24	1.03	3624	3.6
Do.	8/6 & 9/4				3609	3.0
Do.	5/18, 5/28, 8/6, & 9/4				3294	1.3
1954:						
None	-				3111	13.5
Ziram 1 1/2-100	5/28				3205	12.5
Do.	7/9 & 8/26	5/26/55	3.52	2.93	3522	2.5
Do.	5/28, 7/9, & 8/26				3360	1.2

In 1951, all 3 fungicides gave commercial control of the fruit rot, but it was not possible to determine whether the early or the late application was more effective. It was decided to limit subsequent tests to ziram, because captan caused injury to apple foliage and pear fruits at Hood River. Copper fungicides had caused damage to both Yellow Newtown apples and Anjou pears in this district in previous tests.

Both rainfall and fruit rot incidence were low during 1952. The lack of rainfall after June indicates the fruits that rotted were infected earlier than July. Prior to 1951 spray tests to control bull's-eye rot at Hood River were rarely started before August, and it is possible that some fruit developed latent infections before the fungicides were applied. This would explain why a fungicide would give good fruit rot control one year and failed to do so another.

Results of the 1953 spray tests are of special interest. Early spray applications alone reduced the amount of rotted fruit to 3.6 percent as contrasted with 6.3 percent in the fruit from the control plot. Late applications alone also decreased the fruit rot to 3.0 percent. The combination of early and late spray applications was twice as effective in fruit rot control as either alone, as only 1.3 percent of the fruit developed rots.

The data for 1954 show that ziram applied in May had little effect on fruit rot. Extended rains did not occur until August; so the July application probably could have been omitted without seriously affecting rot control. The spray put on during August was applied at a critical time, as shown by the marked reduction in fruit rot from the mid-season application. According to weather records at Hood River, 1954 was an average year, and the spray tests in 1954 are indicative of the fruit rot control possible during years when conditions favor late-season infections.

CONTROL RECOMMENDATIONS

The variations in raintall from year to year make it difficult to establish a fixed spray schedule for control of bull's-eye rot. The following program is suggested:

1. Apply ziram at the rate of 1 1/2 pounds to 100 gallons of water with the first cover spray for codling moth control. This spray is applied to prevent early fruit infections that would remain latent but develop into rot later.
2. A second, and more important, application of ziram (1 1/2 pounds to 100 gallons of water) should be made before fall rains. If tree props prevent spray machinery from being moved through the orchard at a later date, the fungicide should be combined with the last spray for codling moth control.
3. One or more additional ziram sprays may be advisable if excessive rainfall occurs during the season. A grower must judge the necessity for using additional cover sprays by the seasonal weather conditions, the amount of active spore material present in the orchard, and the past history of his orchard with respect to fruit rot.

DISCUSSION

Most of the data on control of bull's-eye rot at Hood River have been obtained from tests on Yellow Newtown apples, starting as far back as 1928. Less extensive tests on Spitzenberg and Ortley apples, and Anjou, Easter, and Winter Nelis pears, gave results similar to those obtained with Yellow Newtown apples. Several outstanding cases of control of bull's-eye rot on both apples and pears have been observed at Hood River since ziram was first used commercially for this purpose in 1952.

Spray tests were rarely started before August in the early work, and severe fruit damage often occurred from the use of copper fungicides on pears or yellow apple varieties. A number of fungicides have been found to control bull's-eye rot. They include ziram, captan, dichlone and several copper compounds. Ziram is being recommended at Hood River because of its safety on apples and pears in mixed plantings, its compatibility with most insecticides used, and its value in scab control during the early season. Other fungicides may have special merits in districts growing less spray sensitive varieties than the Anjou pear or yellow apple varieties. Dusts applied by airplane are sometimes used when ground equipment cannot be pulled through the orchard. Dusts seem to be much less effective for fruit rot control than sprays applied by ground equipment

The results of spray tests from 1951-54 (Table 2) indicate that latent infections may occur at a very early date in apple fruits. This concept is new for pome fruit infections, although a similar development by related fungi in banana (10) and tomato (4) fruits has been shown to occur.

Severe damage to trees at Hood River during the winters of 1949 and 1950 caused a marked

increase in bull's-eye rot. It is of interest to note in Table 1 that no repacking of fruit was necessary after 1952, the year that ziram was first used commercially to control the fruit rot at Hood River. Fruit is generally shipped without repacking if the average total rot is less than 2 percent, with a maximum of not over 4 percent.

The November freeze of 1955 and subsequent low winter temperatures in early 1956 again caused considerable damage to apple and pear trees. Most of the injured bark is already infected by the perennial canker fungus, and in many cases acervuli will sporulate with the first heavy rain. If a wet season occurs during 1956, and particularly if rainfall is heavy and extended during the harvest period, bull's-eye fruit rot will be of major importance. Growers could profitably consider the application of one or two additional late ziram sprays because of the increased source of inoculum in orchards throughout the Pacific Northwest.

Literature Cited

1. Brien, R. M. 1932. "Delicious spot" on apples due to Gloeosporium perennans. New Zealand Dept. Agr. Jour. 45: 215-218.
2. Cordley, A. B. 1900. Apple-tree anthracnose; a new fungous disease. Oregon Agr. Exp. Sta. Bull. 60: 8 pp.
3. Fisher, D. F. 1925. A new apple-rot disease (Bull's-eye rot -- caused by Gloeosporium, Zeller & Childs.) Washington State Hort. Assoc. Proc. 21: 44-48.
4. Fulton, J. P. 1948. Infection of tomato fruits by Colletotrichum phomoides. Phytopath. 38: 235-245.
5. Heald, F. D. 1931. Bull's-eye rots of apples in Washington. Wenoka Arrowhead (Wenatchee, Wash.) 3: 1, 6.
6. Kienholz, J. R. 1939. Comparative study of the apple anthracnose and perennial canker fungi. Jour. Agr. Res. 59: 635-666.
7. Kienholz, J. R., R. H. Robinson, and E. S. Degman. 1949. Reduction of pear rots in Oregon by the use of a chemical wash. Oregon Agr. Exp. Sta. Circ. Inform. 460: 7 pp.
8. Lawrence, W. H. 1904. Blackspot canker. Washington Agr. Exp. Sta. Bull. 66: 35 pp.
9. Roberts, J. W. and L. Pierce. 1918. Apple bitter rot and its control. U. S. Dept. Agr. Farm. Bull. 938. 14 pp.
10. Simmonds, J. H. 1941. Latent infections in tropical fruits discussed in relation to the part played by species of Gloeosporium and Colletotrichum. Proc. Roy. Soc. Queensland. 52: 91-120.
11. Taylor, G. G. and R. M. Brien. 1943. Ripe spot of apples (Neofabraea malicorticis.) New Zealand Jour. Sci. & Tech. Sect. A. Agr. 25: 63-72.
12. White, E. W. 1925. Northwestern apple tree anthracnose canker and fruit rot. Oregon Agr. Exp. Sta. Ext. Ser. Circ. 220: 1-3.
13. Zeller, S. M. and Leroy Childs. 1925. Perennial canker of apple trees. (A preliminary report.) Oregon Agr. Exp. Sta. Bull. 217: 17 pp.

HORTICULTURAL CROPS RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE, U. S. DEPARTMENT OF AGRICULTURE, HOOD RIVER, OREGON

DOES POST-TREATMENT STORAGE OF WHEAT SEED INCREASE THE EFFECTIVENESS OF FUNGICIDES AGAINST SMUT?[1]

Laurence H. Purdy[2]

Holding treated wheat seed for certain prescribed periods before planting is recommended as being essential to maximum control of bunt where certain treating materials are used. The length of the storage period generally recommended by manufacturers of such materials as Ceresan M, Panogen, Agrox, and Mema, may range from 24 to 48 hours. Recently Machacek[3] presented experimental evidence that for maximum effectiveness certain organic mercury seed disinfestants such as Ceresan M, Panogen, and Agrox require a minimum storage period of 6 days. In these same experiments no post-treatment storage was required for formulations of hexachlorobenzene and pentachloronitrobenzene. Because of these divergent requirements expressed with regard to post-treatment storage, investigations were undertaken to determine whether post-treatment storage of wheat seed is essential to effective control of bunt in the Pacific Northwest.

Materials and Methods

These studies were made with Elgin winter wheat and Red Bobs spring wheat. The seed was treated in units of 250 mls by a method previously described.[4]

The following materials were tested:

Agrox -- 6.7% phenylmercuriurea
Anticarie -- 40% hexachlorobenzene
Ceresan D -- 2.8% ethyl mercury 2, 3-dihydroxy propyl mercaptide and 0.6% ethylmercury acetate
Ceresan M -- 7.7% N-(ethylmercuri)-p-toluenesulfonanilide
Mema -- 11.4% 2-methyoxyethylmercuryacetate
Panogen 15 -- 2.2% cyano (methylmercuri) guanidine
Velsicol Emmi -- 10.34% 1,4,5,6,7,7-hexachlor-N-(ethylmercuri) bicyclo [2.2.1] hept-5-ene-2, 3-dicarboximide

This study was extended over the 3-year period 1954-1956. In the first 2 years the treated seed was divided into two lots, one of which was stored in a paper bag while the other was stored in a cloth bag. In the 1956 experiment only paper bags were used to store the treated seed. Each day for 14 days some seed was removed from the storage containers and planted in the field. For comparison with the stored samples one sample of each treatment was planted within a half-hour after application of the fungicide.

Four separate tests were made using randomized single 5-foot row plots with 3 replications per treatment. One was a winter wheat test seeded in the fall of 1954; one was a greenhouse test in 1955; and the other 2 were spring wheat tests, one in 1955 and the other in 1956. The smut percentages recorded were based on counts of smutted and total heads.

---

[1] Cooperative investigation of the United States Department of Agriculture, Agricultural Research Service, Field Crops Research Branch and the Agricultural Experiment Stations of Idaho, Oregon and Washington.

[2] Pathologist, Field Crops Research Branch, Agricultural Research Service, United States Department of Agriculture, Pullman, Washington.

[3] Machacek, J. E. 1954. Co-operative seed treatment trials -- 1953. Plant Dis. Reptr. 38: 169-172.

[4] Purdy, Laurence H. 1955. Regional seed treatment tests for the control of seed-borne and soil-borne common smut of winter wheat in the Pacific Northwest. Plant Dis. Reptr. 39: 844-849.

Table 1. The effect of post-treatment storage in paper and cloth bags on smut control in fall-planted Elgin wheat, 1954.

Treatment Fungicide	Rate[a]	Days Stored Before Planting and Percent Smut											
		0[b]	1	2	3	4	5	6	7	8	9	10	14
Stored in paper bags													
Check (untreated)		88	94	73	43	75	58	60	68	85	80	83	50
Anticarie	1	0	1	0	0	0	0	0	0	1	0	0	0
Agrox	1/2	4	0	1	1	1	1	0	2	1	0	0	0
Ceresan M	1/2	0	1	3	0	0	1	0	0	0	1	0	0
Panogen 15	3/4	2	7	2	0	0	0	0	0	0	0	0	0
Stored in cloth bags													
Check (untreated)		90	94	49	80	83	49	63	75	79	70	55	50
Anticarie	1	0	4	0	0	0	0	0	0	0	0	0	0
Agrox	1/2	5	1	3	0	1	2	0	1	0	0	0	1
Ceresan M	1/2	1	1	6	0	0	0	0	2	0	2	0	1
Panogen 15	3/4	0	9	1	0	1	0	0	0	0	0	0	0

Table 2. The effect of post-treatment storage in paper and cloth bags on smut in greenhouse planted Red Bobs spring wheat, 1955.

Treatment Fungicide	Rate[a]	Days Stored Before Planting and Percent Smut											
		0[b]	1	2	3	4	5	6	7	8	9	10	14
Stored in paper bags													
Check (untreated)		85	63	64	96	100	100	94	98	95	86	0	0
Anticarie	1	2	0	0	0	0	2	0	0	0	0	0	0
Agrox	1/2	2	2	0	3	0	48	9	4	26	0	0	0
Ceresan M	1/2	0	0	0	0	0	6	1	7	15	7	0	0
Panogen 15	3/4	0	0	0	0	5	0	3	3	0	0	0	0
Stored in cloth bags													
Check (untreated)		94	88	68	84	95	100	95	97	87	93	0	0
Anticarie	1	0	0	0	2	0	0	0	0	0	0	0	0
Agrox	1/2	0	0	0	0	0	8	6	5	2	0	0	0
Ceresan M	1/2	0	0	0	0	0	9	2	0	0	0	0	0
Panogen 15	3/4	0	0	0	0	1	0	0	0	0	0	0	0

For footnotes see Table 4

Table 3. The effect of post-treatment storage in paper and cloth bags on smut control in field-planted Red Bobs spring wheat in 1955.

Treatment Fungicide	Rate[a]	Days Stored Before Planting and Percent Smut											
		0[b]	1	2	3	4	5	6	7	8	9	10	14
Stored in paper bags													
Check (untreated)		8	49	86	84	46	88	83	77	59	95	78	75
Anticarie	1	0	0	0	0	0	0	0	5	0	0	0	0
Agrox	1/2	3	16	7	10	10	8	21	14	4	2	4	11
Ceresan M	1/2	0	0	1	0	0	0	0	0	0	0	0	0
Panogen 15	3/4	0	0	0	0	0	0	0	0	0	0	0	0
Stored in cloth bags													
Check (untreated)		50	94	85	95	69	95	62	86	58	89	82	66
Anticarie	1	1	0	2	0	T[c]	T	1	11	1	1	7	0
Agrox	1/2	3	11	8	10	15	12	10	12	6	6	0	2
Ceresan M	1/2	0	0	0	0	0	0	0	0	1	0	5	0
Panogen 15	3/4	0	0	0	0	0	0	0	0	0	0	0	0

For footnotes see Table 4.

Table 4. The effect of post-treatment storage on smut control in field planted Red Bobs spring wheat, 1956.

Treatment Fungicide	Rate[a]	Days Stored Before Planting and Percent Smut										
		0[b]	1	2	3	4	5	6	7	8	9	10
Check (untreated)		85	83	90	75	95	68	78	80	83	68	90
Panogen 15	3/4	T[c]	0	0	0	T	0	0	0	0	0	T
Ceresan M	1/2	0	0	0	0	0	0	T	0	0	0	0
Anticarie	1	T	0	T	0	0	0	0	0	0	0	0
Mema	1/4	T	2	0	0	0	T	T	0	0	0	T
Velsicol Emmi	1/2	0	0	0	0	0	0	0	0	0	0	0
Ceresan D	3/4	0	0	0	0	0	0	0	0	0	0	0

[a]Rate in ounces per bushel.
[b]Planted 1/2 hour after treatment.
[c]Trace, less than 1/2 percent.

Results

The results obtained from the fall seeding in 1954 are presented in Table 1. These data show that good control was obtained with all four materials tested and that post-treatment storage caused little or no significant increase in the effectiveness of the fungicides. Furthermore, there was no differential effectiveness of the materials, whether the seed was stored in paper or cloth bags. The reason for the relatively poor performance of Panogen after one day's storage is not apparent, since it gave good control on all other planting dates, including the one in which no storage period was involved.

Results from the greenhouse test (Table 2) were comparable to those obtained from the fall seeding (Table 1). Good control was obtained with Anticarie and Panogen 15 throughout the test, while control with Ceresan M and Agrox was erratic.

Data obtained from the 1955 field planting of Red Bobs spring wheat are summarized in Table 3. Consistently good control was provided by Ceresan M and Panogen 15 while control with Anticarie was somewhat variable, as it provided good control on all but two dates of seeding. Smut control with seed stored in the cloth bags was comparable to that obtained with seed stored in paper bags, with the possible exception of the Anticarie treatment. Control was relatively poor in the plantings of Anticarie-treated seed stored in cloth bags 7 and 10 days. However, this was the only instance of poor control with this material. In this test the performance of Agrox was unsatisfactory, regardless of the post-treatment storage period.

The results of the 1956 field-planted spring wheat test are presented in Table 4. In addition to Ceresan M, Panogen, and Anticarie, three other materials were included. These were Mema, Velsicol Emmi, and Ceresan D. Agrox was not included in this test because of its unsatisfactory performance in previous tests and in other seed treatment tests. Since the other tests reported here apparently showed no difference in paper and cloth bag storage, only paper bags were used in this test. As shown in Table 4, all treatments gave good control, regardless of length of post-treatment storage.

Storage after treatment with any one of the seven materials tested will not increase the effectiveness of that material in controlling wheat smut, according to the data presented here.

UNITED STATES DEPARTMENT OF AGRICULTURE, AGRICULTURAL RESEARCH SERVICE, AND AGRICULTURAL EXPERIMENT STATIONS OF IDAHO, OREGON, AND WASHINGTON, COOPERATING

AEROBIOLOGY OF RUST SPORES AND EPIDEMIOLOGY OF WHEAT RUSTS IN KANSAS IN 1955 [1]

S. M. Pady and C. O. Johnston [2]

Cereal rusts are an annual problem in Kansas and their epidemiology is carefully followed, especially in the case of leaf rust, Puccinia rubigo-vera (DC.) Wint. var. tritici (Erikss.) Carl., and stem rust, Puccinia graminis var. tritici (Erikss. & E. Henn.) Guyot, of wheat. In recent years the rust diseases have been highly variable. A major stem rust epidemic was averted in 1953 only by the advent of extremely high temperatures in June, and the loss of wheat from stem rust was estimated to be 1.5 percent. On the other hand, leaf rust was unusually scarce that year, being present only as a trace in most fields. In 1954 a minor epidemic of stem rust caused a 2.5 percent loss and was again checked by a heat wave in June. Leaf rust that year was heavy in central Kansas and caused a 2.0 percent loss for the State. In 1954 aerobiological studies were combined with epidemiology, and the presence of stem rust and leaf rust spores in the air was ascertained daily at Manhattan from May to mid-July and again from September 12 to October 16 [3] Similar studies were undertaken in 1955 and the results are reported here.

Fall and Winter Situation

During the fall of 1954 from September 10 to October 16, the exposed slides showed leaf rust spores to be present on only one day, September 13, and leaf rust infection was extremely rare in the field. Stem rust, on the other hand, was rather abundant both on the slides and in fields. Stem rust spores were present in the air September 13, 20, 23, 24, 28, 29, October 3, 4, 5, 13, 14, 16. Although there was very little volunteer wheat in Kansas in September 1954, there were fairly heavy infections of stem rust in some volunteer fields by October 1, and primary uredia were present on sown wheat in central Kansas.

During October both rusts developed for a time with leaf rust increasing considerably. By the end of October stem rust was heavy on wheat sown in August and early September at Manhattan. With the coming of lower temperatures and a heavy frost November 1, stem rust dried up rapidly and could be found only on dry dead leaves. The spores, however, were nonviable. Leaf rust also began to disappear and was very difficult to find by January 1. From January 1 to April rusts of wheat were not evident in the State.

From the data on rust infections on volunteer and seeded wheat and from the exposed slides, it is evident that rust spores were carried into Kansas in September and October, and they provided abundant inoculum for the wheat fields that lay south of the frost line.

Overwintering

Stem rust apparently did not overwinter in the State, but one case of overwintering of leaf rust was observed in experimental plots of the agricultural experiment station near Manhattan. The infected wheat was sheltered on the north by a hedge and was heavily infected by May 5 when there was little infection elsewhere in the State. There were also some reports of leaf rust overwintering in a few fields of volunteer wheat and wheat sown very early in southeastern Kansas. Conditions were generally unfavorable for overwintering of the two rusts in most localities.

Condition of Crop, Winter and Spring, 1955

The 1955 wheat crop emerged in good condition in most parts of the State. However, in the south central and southwestern counties germination was delayed by fall and winter drought, and the wheat either failed to emerge or made little growth after emergence. Many fields in this area blew out during the high winds and dust storms of March and April. In the central part of the State the wheat plants emerged late and were small and weak throughout the winter and early

---

[1] Contribution No. 622, Serial No. 476, Department of Botany & Plant Pathology, Kansas Agricultural Experiment Station, Manhattan.

[2] Respectively, Head of Department of Botany and Plant Pathology, Kansas State College, Manhattan, and Pathologist, Field Crops Research Branch, Agricultural Research Service, U.S. Department of Agriculture, located at Manhattan, Kansas.

[3] Pady, S. M. and C. O. Johnston. The concentration of airborne rust spores in relation to epidemiology of wheat rusts in Kansas in 1954. Plant Dis. Reptr. 39: 463-466. 1955.

spring. In the north central counties fall conditions were more favorable and the plants made better growth.

The late freeze of March 25 and 26 extended through south central Kansas and most of Oklahoma and Texas. The freeze caused some injury to wheat in Kansas and much more damage in Oklahoma and Texas. In some fields in Texas the plants were frozen back to the crowns, resulting in the formation of new tillers. As a result, ripening was greatly delayed and the crop matured unevenly. One advantageous result of this freeze, however, was a great reduction in the amount of cereal rust inoculum in Texas and Oklahoma at a critical time. After three successive years of severe winter and spring drought, there was enough rain in southern and central Texas in February and March 1955, to produce almost normal rust conditions, and leaf and stem rusts had developed very rapidly. The increase was so rapid that a potential epidemic was in the making by the end of March. The freeze effectively checked development, and rust infections were almost completely eliminated and the rusts never were able to regain the lost momentum and concentration.

In Kansas, wheat had matured early in the central and north central counties because of high temperatures and lack of moisture from May 10 to 24. The plants were only 4 or 5 inches tall in many fields. When rains came May 24 and 25, these plants produced a new crop of late tillers which were flowering when the first heads were mature. Thus many fields in central Kansas had a "two-story" appearance, a lower story of mature heads on short straw and an upper story of late green heads on stems a foot or more taller.

Rust Spores in the Air

Beginning with a few exposures in March, silicone-coated slides were exposed in a wind-vane type spore trap on the roof of Willard Hall, at Kansas State College. Beginning April 9, regular exposures for 2-day periods and observations were made until harvest on the incidence of rust spores in the air, meteorological conditions, and development of the rust in the field. The data for this period are shown graphically in Figures 1 and 2. The first leaf rust was observed on slides exposed April 14, at which time there were warm southerly winds (Fig. 1). Favorable temperatures combined with showers April 11, 13, and 14, provided conditions for infection and by April 23, scattered leaf rust primaries were present in many of the central counties. Frequent showers and warm temperatures were characteristic of the last half of April and the first half of May By May 9 leaf rust was rather general, mostly as primaries, over the central and eastern counties. There was more leaf rust in the eastern part of the State than in any other section.

From May 16 on, leaf rust spores were present daily on the slides and reached a maximum June 1, when southerly winds brought in large numbers. In central Kansas there was considerable late leaf rust on the new growth following the rains of May 24 and 25. In western Kansas only a trace of leaf rust was present in most fields.

A factor that affected the amount of leaf rust was that the wheat crop was 10 days to 2 weeks earlier than normal through April and May and this, along with drought in many parts of the State, caused the wheat to ripen before leaf rust could become heavy. During June, high numbers of leaf rust spores in the air at Manhattan reflected the development of rust on the late growth of wheat in central Kansas, and they were present in the air until almost harvest (Fig. 1).

The number of leaf rust spores caught on slides was much lower in 1955 than in 1954, with a peak of 53,000 on June 1 (Fig. 1) compared with 167,000 on June 8, 1954. The lower frequency of spores in the air reflects the decreased amount of leaf rust in the State compared with the previous year. Leaf rust damage thus was considerably less than in 1954 and has been estimated to be 1 percent, compared with 2 percent the previous year.

Prior to May 23, stem rust spores were present only in trace amounts five times between April 13 and May 23 (Fig. 2). From that date stem rust spores were present on the slides every day through June and July with a peak reached just about the time harvest began locally and continuing in fairly large numbers until mid-July. The first stem rust uredia were observed in the field in eastern Kansas on May 28. By June 3 traces were present in most fields in eastern Kansas, and by June 14, stem rust was general in the eastern two-thirds of the State. Harvesting had commenced in southern and western Kansas. Except in areas where regrowth had taken place, there was little opportunity for further development of stem rust. The absence of substantial rains from June 6 to 30 in central Kansas and the presence of high temperatures over the State from June 16 on (Fig. 2) also were factors that helped check development of stem rust. Even in fields where regrowth had provided sites for further stem rust infections, there was little loss from stem rust. The prevalence of stem rust spores in the air was considerably less than in the previous year. In 1954 the loss from stem rust was 2.5 percent but the estimated

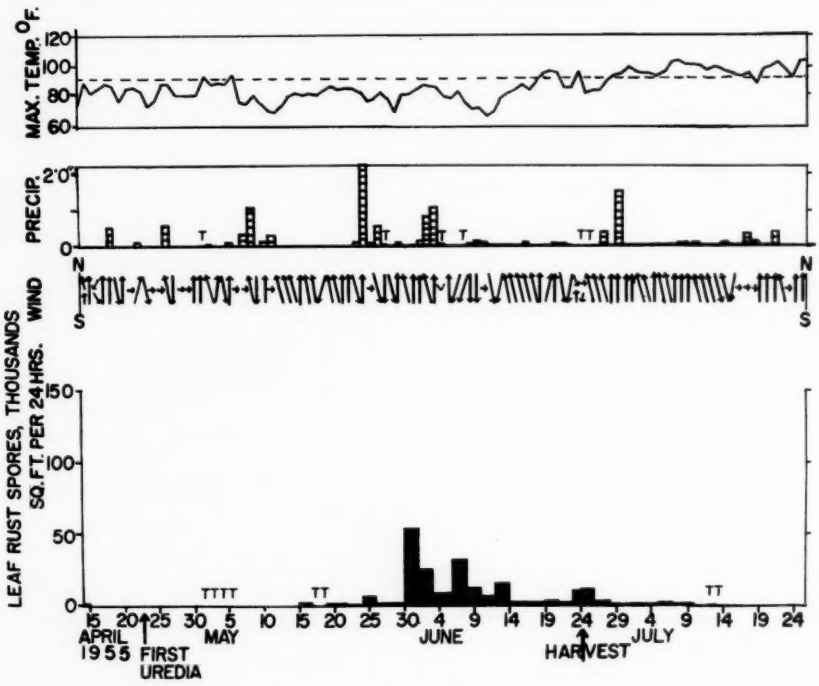


FIGURE 1. Data on maximum temperatures, precipitation, wind direction, and number of leaf rust spores trapped per square foot per day at Manhattan, Kansas, from April 14 to July 25, 1955. Meteorological data obtained from a station on campus.

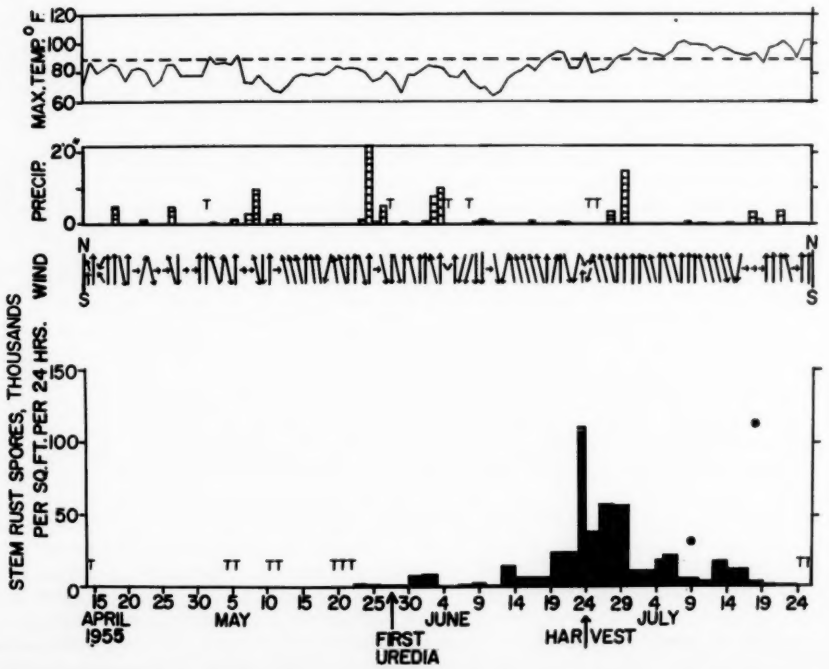


FIGURE 2. Data on maximum temperature, precipitation, wind direction, and number of stem rust spores trapped per square foot per day at Manhattan, Kansas, from April 14 to July 25, 1955. Meteorological data obtained from a station on the campus.

loss for 1955 was considered to be only a trace.

Stem rust spores continued to be present in the air in substantial numbers (Fig. 2) even after harvest was completed and provided abundant inoculum for wheat in the States and Provinces to the North.

Stem rust, Puccinia graminis var. avenae Erikss. & E. Henn., appeared in oat fields in central Kansas early in June and by June 14 nearly all fields were moderately to heavily infected. Toward the end of June such fields frequently were severely lodged due to stem rust. Oat fields in eastern Kansas also were infected, but not so severely as in the central counties. The loss from stem rust of oats has been estimated to be 2 percent for the State. Since the urediospores of stem rust of oats are indistinguishable from those of stem rust of wheat, it is possible that some of the stem rust shown in Figure 2 the last part of June and the first part of July included some oat stem rust.

Summary

Leaf rust, Puccinia rubigo-vera (DC.) Wint. var. tritici (Erikss.) Carl., was rare in Kansas the fall of 1954, both on spore-trap slides and in the field, whereas stem rust, Puccinia graminis var. tritici (Erikss. & E. Henn.) Guyot, was relatively abundant. Conditions were unfavorable for stem rust overwintering, and, with the few exceptions, this was true also for leaf rust. A leaf rust spore shower occurred April 14, 1955, when southerly winds carried in rust spores. The first uredia were present in fields by April 23 and were fairly general by May 15. Leaf rust spores were present in the air daily from May 15, reaching a peak June 1, and decreasing gradually at harvest time (June 24 at Manhattan). Warm dry weather from June 14 on checked further leaf rust damage. The loss of wheat for the State was estimated at 1 percent. Stem rust spores were present in the air in trace amounts in April and May and uredia were first observed May 28. From May 23 on spore showers occurred daily with a peak at harvest time. High temperatures and low rainfall effectively checked stem rust development and the average loss was only a trace.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, KANSAS AGRICULTURAL EXPERIMENT STATION, MANHATTAN, KANSAS, AND FIELD CROPS RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE, U. S. DEPARTMENT OF AGRICULTURE.

YIELD REDUCTION IN WHEAT BY STRIPE RUST

Francis J. LeBeau, Oscar N. Sosa, and A. Fumagalli [1]

The estimation of the loss that can be attributed to rust diseases of wheat is usually difficult. Numerous studies have been made on losses due to stem rust, Puccinia graminis tritici, but little information is available on the losses that can be caused by stripe rust (Puccinia glumarum).

During the past 7 years yield trials and disease data have been obtained for a large number of wheat varieties in the Valley of Quezaltenango (7800 feet elevation) in Guatemala. During the first 5 years Puccinia glumarum appeared only as traces or not at all. In 1954 and again in 1955 stripe rust appeared in the form of severe epidemics especially on the variety Supremo 211. The appearance of the stripe rust epidemic during these 2 years coincided with the rapid increase in acreage of Supremo 211 in the Quezaltenango Valley.

In the epidemic years of 1954 and 1955 sharp drops were observed in the yield of Supremo 211. Each year since 1951 from 25 to 30 acres of this variety have been grown for foundation seed at the sub-station of the Servicio Cooperativo Interamericano de Agricultura in the Quezaltenango Valley. For the years 1952 and 1953 the average yields of Supremo 211 were 33 and 31 bushels per acre, respectively. In 1954 and 1955 this variety yielded only 20 and 21 bushels per acre, respectively. Thus, the yields for 1954 and 1955 were approximately 63 percent and 65 percent of the average yield for the two non-rust years of 1952 and 1953.

While other factors undoubtedly could have been responsible for this sudden drop in yield it is believed that the greatest single factor was the severe epidemics of P. glumarum during 1954 and 1955. This conclusion is supported by the following observations.

During the years 1948 to 1955 the varieties Supremo 211 and Frondoso were frequently used as common checks in variety trails. Thus, parallel yield data from these two varieties are available from at least one trial to as many as ten trials per year during this period. If the yields of the Supremo 211 plots for each year are expressed as a percentage of the yields of the corresponding Frondoso plots, the following percentages are obtained for the years 1948 to 1955: 106, 106, 124, 105, 93, 102, 67, and 66. It is to be noted that the two varieties were quite similar in yield except during the two stripe rust epidemics of 1954 and 1955.

The variety Frondoso is not entirely resistant to P. glumarum but it is much more resistant than Supremo 211, and that difference probably accounted for the better showing by Frondoso relative to Supremo 211 during 1954 and 1955.

The ratner close agreement between these data comparing the yields for Frondoso and Supremo 211, and the sudden drop in yields of Supremo 211 after the appearance of P. glumarum in epidemic proportions, strongly suggest that the main reason for the low yields of Supremo during 1954 and 1955 was the heavy infection by P. glumarum. If this is true, it is therefore indicated that P. glumarum can reduce yields by as much as 30 percent.

SERVICIO COOPERATIVO INTERAMERICANO DE AGRICULTURA DE GUATEMALA

[1] Plant Pathologist, Associate Agronomist, and Assistant Agronomist, respectively, of Servicio Cooperativo Interamericano de Agricultura de Guatemala.

CORRELATION BETWEEN CORN GERMINATION IN LABORATORY COLD TESTS AND STANDS IN THE FIELD

Paul E. Hoppe[1]

Data on the value of corn cold testing were obtained at Madison, Wisconsin, in 1956. Thirty-six lots of 2-year and 3-year old seed, originally treated, and submitted for certification after being tested for germination in standard laboratory tests, were cold tested using the rolled towel technique[2]. The cold tests were made both before and after the seed was adequately re-treated with 50 percent Thiram (Arasan) dust. All tests, including those made subsequently in the field, were in three 50-kernel replications.

The field planting was made May 17 in rows 4 feet long, when corn planting in the area generally was at a peak. A planting of seed retreated with captan (Orthocide) also was included in the field test for a comparison with thiram. Daily temperatures fluctuated considerably following the planting. First seedling emergence occurred in 10 days, which indicated that temperatures had not been especially favorable for a good field cold test.

Results

Data for the laboratory and field tests and the calculated statistical correlations are given in Table 1. Germination in the standard tests averaged 95.3 percent. A comparison of the overall means shows that germination in the laboratory cold tests was much lower than were the stands in the field. The average laboratory cold test germination for all samples as treated by the producers was 34.2 percent while the field stands averaged 78.4 percent. For the thiram re-treated seed the germination was 59.0 and 84.6 percent, respectively, for the laboratory and field tests. Field stands following re-treatment with captan averaged 86.1 percent.

The much less severe field environment than that imposed under controlled conditions in the laboratory cold tests undoubtedly accounted for the differences reported. The data show, however, that the seed lots that were lowest in germination in the laboratory cold tests generally produced the poorest field stands while the better ones in the laboratory tests gave the best field stands (Fig. 1). The relationships are best indicated by the correlation coefficients, of $r = 0.626$ for the laboratory and field comparison of originally treated seed, and $r = 0.673$ for the thiram re-treated samples. These values are highly significant since only 0.418 was required for significance at the 1 percent level.

Discussion

The present study shows that cold tests are useful for spotting mediocre or poor seedlots that pass existing laboratory germination test standards but are likely to produce inferior stands and reduced yields when planted in the field.

For several years the Wisconsin Seed Certification Service has cold tested samples submitted for seed certification, partly for the purpose of advising the producers when seed quality was in question. As a consequence many of the samples in the present study that passed the laboratory germination test for certification but which were found to be of inferior quality in the cold tests, actually were not certified by the decision of the producers themselves. At a meeting of Wisconsin hybrid producers in November 1955, the group went on record as favoring the requirement of cold tests for all seed two or more years old. This procedure is under consideration although the standards to be applied are yet to be decided.

The increased germination shown in this experiment following re-treatment of the old seed supports the contention that all carry-over seed should be re-treated. The field benefits obtained under conditions less severe than those frequently encountered in Wisconsin should leave little question about the importance of good seed treatment.

---

[1]Pathologist, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

[2]Cold Testing Seed Corn by the rolled towel method. Wis. Bul. 507. 1955.

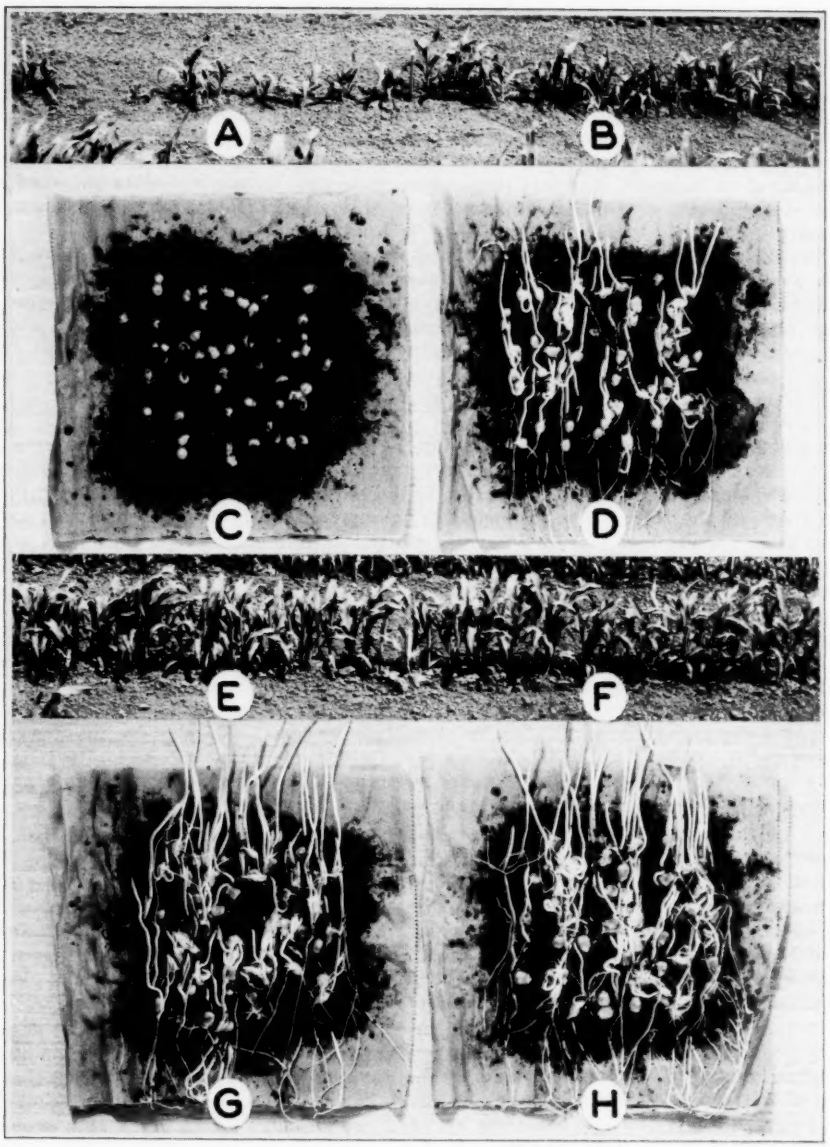


FIGURE 1. Correlation between germinations in laboratory cold tests and subsequent field stands of corn. **A)** Field stand from seed as originally treated by producer. **B)** Field stand from same seedlot following re-treatment with Thiram. **C)** Cold test of seed as treated by producer. **D)** Cold test of re-treated seed. **E)** Field stand from seed as treated by producer and having good germination in the cold test. **F)** Field stand from same seedlot following Thiram retreatment. **G)** Cold test of seed as treated by producer. **H)** Cold test of re-treated seed.

Table 1. Standard laboratory germination, cold test germination and field stands of seed corn, before and after re-treatment with thiram and captan, of seed submitted for certification and originally treated with a fungicide. (Average percent from three 50-kernel replications).

Seedlot	Standard germination	Cold test germination		Field stands		
		Original treatment	Re-treated thiram	Original treatment	Re-treated thiram	Re-treated captan
4001	--	90	95	87	82	84
4544	97	0	6	44	50	69
589	96	89	94	94	93	95
552	89	56	61	55	70	74
496	98	94	99	93	94	93
4542	98	0	3	53	84	87
658	96	6	65	77	77	83
553	89	16	39	61	72	72
745	94	25	64	84	87	87
3632	96	14	58	82	94	89
3631	96	27	56	83	87	82
2688	92	8	8	61	79	78
2687	96	9	55	76	66	88
2565	98	97	97	93	94	96
2557	98	93	94	94	97	89
2539	98	96	98	90	98	96
2461	97	0	64	70	86	83
2459	94	1	54	76	82	78
2380	97	17	42	86	90	88
2294	96	18	66	85	89	89
2291	93	11	45	83	80	89
2236	95	47	60	92	94	97
1818	92	24	54	70	79	86
991	94	0	37	63	74	75
863	96	10	44	67	73	85
855	97	29	64	84	92	95
497	98	94	99	91	95	91
467	98	94	99	94	98	91
749	93	33	52	83	83	90
802	96	25	65	83	83	90
1816	94	31	55	81	80	83
1672	96	1	26	41	80	70
1414	95	27	65	87	87	92
1266	96	40	60	87	91	93
1185	97	3	49	71	85	84
1171	89	4	32	81	80	87
Means	95.3	34.2	59.0	78.4	84.6	86.1

Correlations:	Coefficient	Significant at 1% level
Cold tests and field stands of originally treated seed	.636	.418
Cold tests and field stands of re-treated seed (Arasan)	.673	.418

FIELD CROPS RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE
U. S. DEPARTMENT OF AGRICULTURE AND WISCONSIN AGRICULTURAL EXPERIMENT STATION

PATHOGENICITY OF ROOT ROTTING FUNGI OF OATS[1]

S. F. Hassan[2]

SUMMARY

During the years 1946 and 1947, a survey was made of the organisms causing root rots and seedling blight of oats in Minnesota. Most of the isolates from basal parts of diseased plants were species of Fusarium and Helminthosporium, primarily Fusarium roseum f. cerealis and Helminthosporium victoriae. Other fungi obtained were Rhizoctonia solani, Pythium irregulare, Alternaria spp., Phoma terrestris, Trichoderma spp., Rhizopus spp., and Aspergillus spp. None of the isolates belonging to the last five genera were parasitic. A few isolates of F. roseum f. cerealis were virulent on seedlings of Clinton oats in the greenhouse. Isolates of H. victoriae differed greatly in their pathogenicity on Vicland oats and on the basis of the degree of virulence, three physiologic races could be readily distinguished. An isolate of R. solani attacked eight varieties of oats as well as wheat, rye, barley, and soybeans. Of the 32 isolates of P. irregulare, one was very virulent on seedlings of oats at high temperatures. High soil temperature favored the development of seedling blights caused by H. victoriae and R. solani, whereas it had little or no effect on the Fusarium isolates tested.

Seedling blight and root rot are common diseases of oats in Minnesota, although they were not reported destructive until Helminthosporium victoriae Meehan & Murphy became prevalent on Vicland and Tama varieties. From 1946 through 1948, H. victoriae caused enormous losses to oats, not only in Minnesota, but throughout the oat growing region in the United States (4, 5).

Root rot and seedling blight of oats are caused by many pathogens and the diseases have been reported from many different parts of the world. Simmonds (9) has reported Fusarium culmorum (W. G. Sm.) Sacc. as causing severe seedling blight and root rot in Western Canada and in the North Central and Pacific Coast States of the United States. Bennett (1) in England found that F. culmorum and F. avenaceum (Fr.) Sacc. caused severe damage to adult oat plants. Root rot of oats, caused by Rhizoctonia solani Kuehn, has been reported as important in Australia (3, 8). Other investigators (12) have reported that Pythium debaryanum Hesse caused severe seedling blight and root rot of oats. Downie (2) reported that Pythium spp. frequently cause seedling blight or damping off in oats when grown in the greenhouse. O'Brien and Dennis (7) have published a rather detailed acount of a destructive root disease of oats in Scotland caused by Helminthosporium avenae Eidam.

Although root rots and seedling blight of oats are common diseases in Minnesota, no extensive studies have been made on the relative importance of the different pathogens, or under what conditions they are most destructive. Therefore studies were made on the prevalence of organisms that cause root diseases of oats grown commercially in Minnesota. Attempts also were made to determine what factors influence the development of different organisms. Obviously such information would be of material help, particularly in testing for root rot resistance in the disease gardens.

---

[1] Paper No. 3584, Scientific Journal Series, Minnesota Agricultural Experiment Station.

Part of a thesis presented to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, June, 1949.

The writer is greatly indebted to a number of peoplefor help and advice during the experimental work and in preparing the manuscript; he wishes to thank especially Dr. E. C. Stakman and Mr. M. B. Moore for their valuable help and guidance during the course of this study.

[2] Formerly graduate student, Department of Plant Pathology and Botany, University of Minnesota.

Table 1. Fungi isolated from 1500 pieces of the basal parts of diseased plants and 1200 seeds of several varieties of oats collected from St. Paul and southern Minnesota, and the pathogenicity of the isolates tested[a].

Organism isolated	Percentage isolates from: Seeds	Percentage isolates from: Basal parts of plants	Number isolates tested for pathogenicity	Percentage isolates pathogenic to oat seedlings
Helminthosporium spp.	9.5	34.8	120	47.5
Fusarium spp.	1.4	36.0	100	20.0[b]
Alternaria spp.	13.2	4.2	11	0
Pythium irregulare	0	2.1	32	3.1
Rhizoctonia solani	0	0.8	12	100.0
Phoma terrestris	0	0.4	6	0
Trichoderma spp.	0	0.4	7	0
Rhizopus spp.	0.6	0.3	4	0
Aspergillus spp.	0.8	0.2	2	0
Clean	74.5	20.8	-	-

[a] Tests were made on Clinton, Mindo and Vicland varieties of oats.

[b] Only mildly pathogenic.

MATERIALS AND METHODS

All isolations were made from diseased root, crown, and mesocotyl tissues of oat plants and from seeds collected in 1946 and 1947 from various fields in southern Minnesota. The following methods were used: 1) For the isolation of Pythium: small pieces of diseased tissue were washed in sterile water and then placed under non-nutrient agar. Within 48 hours, any Phycomycete growing through the agar was examined with the microscope and pure cultures were isolated. This is a modification of Meredith's (6) and Sleeth's (10) method of obtaining bacteria-free cultures of Phycomycetes. 2) For the isolation of fungi other than Pythium: pieces of diseased tissue, or seed, were surface sterilized in a 1:1000 solution of bichloride of mercury for 1 to 2 minutes, washed with a 0.5% sodium hypochlorite solution and plated on potato-dextrose agar.

Steam-sterilized soil consisting of 2 parts loam to 1 part sand was used in 4-inch pots in all pathogenicity tests in the greenhouse. Inoculum of Pythium was grown on potato-dextrose broth. This inoculum was applied by cutting small pieces of the mycelial mat and placing them in the pots at the seed level. Inoculum of other species of fungi was grown on a grain medium in 250-ml. Erlenmeyer flasks. Equal amounts of this inoculum were added to each pot at the seed level before planting.

All examined seed lots of Vicland and Tama oats were heavily infected with Helminthosporium victoriae, hence these seed lots were treated with hot water as follows: The seed

Table 2. Pathogenicity of isolate R-57 of Rhizoctonia solani to oats, wheat, barley, rye, and soybeans, grown in steamed soil in the greenhouse at about 26° C[a].

Host	Percentage Non-emergence		Percentage Post-emergence killing	
	Check[b]	Inoculated[c]	Check	Inoculated
Oats, Andrew	2	35	2	63
Bonda	0	7	0	50
Clinton	0	21	0	59
Garry	4	20	0	81
Gopher	2	20	0	85
Huron	4	19	0	75
Mindo	0	27	2	67
Vicland	6	29	0	78
Wheat, Mida	0	28	0	4
Barley, Wisc. 38	14	7	0	17
Rye, Imperial	14	23	2	54
Soybeans, Manchu Wisc. 3	15	80	0	20

[a] Deviation of several degrees frequently occurred.

[b] Fifteen seeds were planted in each of the 3 replicates.

[c] Based on the percentage of emergence in the check.

was pre-soaked in tap water at room temperature for 6 to 8 hours, dipped in water at 120° F for 2 to 3 minutes, soaked in hot water at 127° F for 20 minutes and then spread on paper towels to cool and dry. This method left at most only 5 percent diseased seeds and caused no seed injury. Seeds of varieties not susceptible to H. victoriae were disinfected by soaking in a 1:1000 solution of New Improved Ceresan for 30 minutes, followed by a 2-hour wash in running water.

All the pathogenicity tests were made on autoclaved sandy loam, 2 parts loam and 1 part sand in sterilized 4-in. pots. Twenty-five seeds per pot were planted 1/2 to 3/4 in. deep. Inoculum was grown on wheat medium or on potato-dextrose broth in 250 cc Erlenmeyer flasks for 1 to 2 weeks and equal amounts were added to each pot at the seed level before planting.

The degree of pathogenicity was based mostly on the severity of pre- and post-emergence killing. In cases where no killing occurred, the amount of stunting or the number of lesions were taken into consideration.

RESULTS

Fungi Isolated from Oats

A summary of the fungi isolated from the basal parts of oat plants and from oat seeds is given in Table 1. Helminthosporium spp. and Fusarium spp. predominated among isolates. The Helminthosporium isolates were identified as H. victoriae with the exception of four isolates of Helminthosporium pedicellatum Henry and a few of the Helminthosporium sativum group. Fusarium roseum (Lk.) Snyder & Hansen comprised most of the Fusarium spp. isolated.

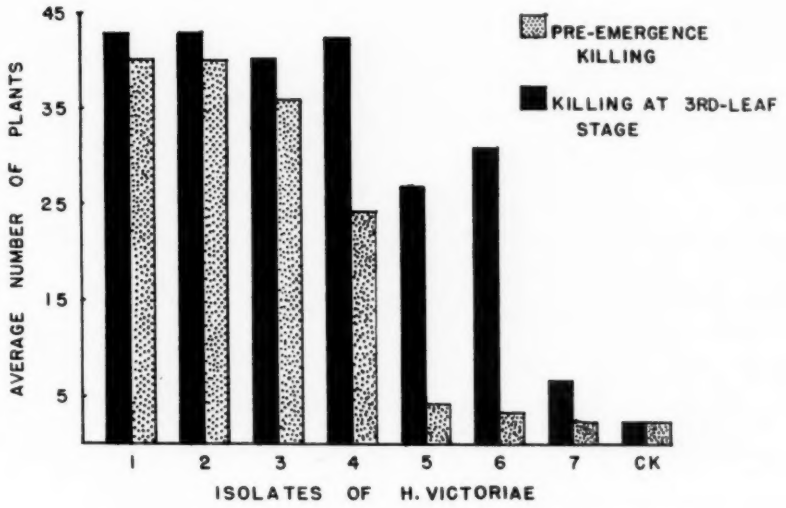


FIGURE 1. Average number of plants killed before emergence and at third-leaf stage of seven varieties of Victoria parentage, inoculated with seven isolates of Helminthosporium victoriae.

About 25 samples of Tama and 25 samples of Vicland seed were collected from various parts of Minnesota during 1946 and 1947. H. victoriae was isolated from 80 percent of the 1946 seed and 20 percent of the 1947 seed.

One sample of Gopher oats from the 1946 crop had about 15 percent of the seeds infected with H. avenae.

Pathogenicity of Root-rotting Fungi

Representative isolates were selected on the basis of their source and culture type and tested in the greenhouse for pathogenicity on seedling plants. Trichoderma spp., Rhizopus spp., Aspergillus spp., and Phoma terrestris were non-pathogenic to Clinton, Mindo, and Vicland oats. Alternaria spp., although frequently isolated from seeds, were non-pathogenic when grown at relatively low (average about 15° C) and high (average about 26° C) temperatures. This was also true of the 32 isolates of Pythium isolated from field-grown plants; however, an isolate of Pythium from oats, grown in non-autoclaved soil in the greenhouse, was highly virulent at 35° C when grown in control-temperature tanks.

Fusarium spp. were most frequently isolated. A pathogenicity test was made of 100 selected Fusarium isolates on oat varieties Bonda, Clinton, Marion, and Mindo in the greenhouse at an average temperature of 21° C; only 20 were found to be pathogenic. All varieties were equally resistant. Some isolates caused slight pre-emergence killing, some slightly stunted the seedlings but none caused any post-emergence killing. One of the slightly pathogenic isolates was tested at seven soil temperatures ranging from about 15° to 36° C and four isolates were tested on seedlings subjected to high soil moisture and low light intensity. All of these treatments failed to increase the pathogenicity of the isolates tested.

Helminthosporium spp. were the next most frequent isolates obtained from the basal parts of diseased plants. One hundred and twenty selected isolates of Helminthosporium spp. were tested at relatively high temperatures on Vicland oats. They showed a wide range of pathogenicity, some causing 100 percent killing of the seedlings while others were non-pathogenic. Therefore, five classes were set up as follows:

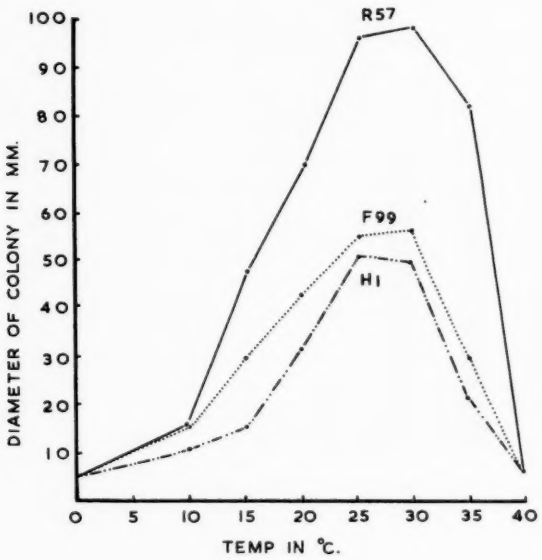


FIGURE 2. Relative radial growth of isolates of Rhizoctonia solani (R-57), Fusarium roseum f. cerealis (F-99), and Helminthosporium victoriae (H-1), on potato-dextrose agar, after 54 hours.

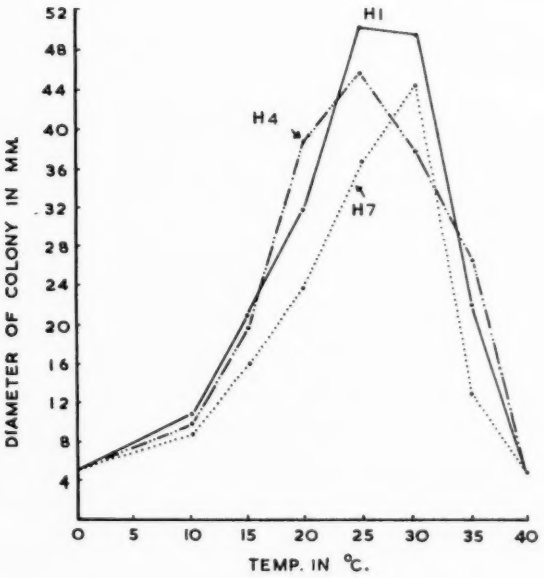


FIGURE 3. The radial growth of three isolates of Helminthosporium victoriae (H-1, H-4, H-7) at different temperatures, on potato-dextrose agar, after 54 hours.

Class 0 -- 100 percent emergence compared to check; no root rot symptoms; non-pathogenic.
Class I -- About 80 to 90 percent emergence; up to 25 percent of emerged plants diseased and killed.
Class II -- 75 to 90 percent emergence; 50 to 75 percent of emerged plants diseased and killed.
Class III -- 50 to 75 percent emergence; 60 to 75 percent of emerged plants diseased and killed.
Class IV -- 10 to 50 percent emergence; 75 to 100 percent of emerged plants diseased and killed.

Of the 120 isolates tested there were 43 in Class 0, 30 in Class I, 18 in Class II, 18 in Class III, and 11 in Class IV. All the pathogenic isolates (Class I to IV) were identified as H. victoriae. The four isolates of H. pedicellatum and the isolates of the H. sativum group proved non-pathogenic on Vicland and Clinton oats, Wisconsin 38 barley, and Mida wheat.

The striking differences in the virulence of isolates of H. victoriae, observed in the preliminary tests, suggested the possibility of parasitic races in this species. To test this further, seven varieties of oats, Boone (C.I. 3305), Cedar (C.I. 3314), Forvic (C.I. 4164), Garry (R.L. 1692), Neosho (C.I. 4141), Osage (C.I. 3991), and Vicland (C.I. 3611), all susceptible to H. victoriae, were tested against seven representative isolates of this fungus. On the basis of the results (Fig. 1), these seven isolates can be grouped roughly into three parasitic or physiologic races: 1) isolates 1, 2, 3, and 4 with a high percentage of pre- and post-emergence killing; 2) isolates 5 and 6 with moderate killing; and 3) isolate 7 with little or no killing.

Eleven isolates of Rhizoctonia solani obtained from field-grown plants, and one isolate, R-57, obtained from oat seedlings grown in the greenhouse in field soil, were tested in greenhouses at soil temperatures of about 15° and 26° C, respectively on Clinton and Vicland seedlings. During the day it was not uncommon to have soil temperatures vary a few degrees. Isolate R-57 caused 50 percent pre- and post-emergence killing by attacking and rotting the coleoptile and the stem at the ground level in the 1- or 2-leaf stage. Although this fungus was not isolated so frequently as other fungi, isolate R-57 was selected for further studies because of its virulence. The 11 isolates of R. solani from field-grown plants were discarded since all were non-pathogenic or only slightly pathogenic to oats in these tests.

In preliminary tests both Clinton and Vicland were equally susceptible to isolate R-57. Therefore, a number of common varieties of oats, wheat, barley, rye, and soybeans were tested against isolate R-57 in the greenhouse at relatively high temperatures. The results, given in Table 2, indicate that isolate R-57 can attack several varieties of oats as well as wheat, barley, rye, and soybeans. In general, the symptoms on cereals were similar, although injury was not so severe on wheat and barley as on oats and rye. In wheat and barley only the coleoptile was attacked and the seedlings were stunted but continued to grow. Rye was very sensitive and developed symptoms in 3 days after emergence, the seedlings dying within 2 days thereafter. It took 1 or 2 weeks to kill oat seedlings. These results indicate that isolates of R. solani differed greatly in their virulence on oats and that at least one isolate was strongly pathogenic on varieties of oats and rye.

The Effect of Temperature on the Pathogenicity of Root-rotting Fungi

Effect of temperature on the growth in culture of one isolate each of Helminthosporium victoriae (H-1), Rhizoctonia solani (R-57), and Fusarium roseum f. cerealis (F-99) was determined at 9 temperatures with 5-degree intervals, ranging from 0° to 40° C, inclusive. The results are given in Figure 2. The optimum temperature for the growth of all three fungi was between 25° and 30° C. There was little difference in the growth of the three isolates of H. victoriae (H-1, H-4, and H-7) representing the three physiologic races with high, moderate, and low degrees of virulence, respectively. It may be noted that the very virulent isolate H-1 grew almost equally well at both 25° and 30° C while the less virulent isolates H-4 and H-7 grew best at 25° and 30° C, respectively. Further, their ability to grow at 25° C appears directly correlated with the degree of their pathogenicity (Figure 1).

Greenhouse tests, in triplicate, with 25 seeds per pot, indicated that soil temperatures above 31° C caused a significantly higher rate of killing of oat seedlings by isolates 1 and 4 of H. victoriae and isolate R-57 of R. solani. It was the general observation of the writer in the preliminary studies, and in the field, that at low soil temperatures of about 15° C or lower

there was considerably less damage to seedlings due to these pathogens. It was concluded that seedling blights of oats caused by H. victoriae and R. solani were favored by higher temperatures.

DISCUSSION

A survey of the fungus flora of the diseased roots of oats from various parts of Minnesota indicated that Fusarium spp. and Helminthosporium spp. were commonly associated with root rots. Among these, H. pedicellatum was obtained in four cases for the first time from oats. The common association of Fusarium spp. with diseased roots led to the belief that Fusarium was one of the principal pathogens involved. It was shown that normally these isolates of Fusarium caused very little or no injury to oat seedlings. Downie (2) obtained similar results. It must be pointed out, however, that the inability to attack seedlings does not necessarily indicate an inability to attack mature plants. Fusarium spp. may play an important part in the complex of pathogens which attack and damage the roots of oat plants. On the basis of the results of the tests reported in this paper, it can be concluded that these Fusarium isolates are unimportant as a cause of seedling blight of oats when they are the only pathogens involved. Further work is necessary to establish their respective importance in the complex of pathogens involved in root rot of oats.

Studies on pathogenicity in the greenhouse proved that Helminthosporium victoriae was chiefly responsible for root rot and seedling blight of commercial varieties of oats having Victoria parentage. Such varieties were extensively grown in 1946 and 1947 and the high frequency at which this species was isolated from diseased plants collected during these years adds support to the findings in the greenhouse.

Isolate R-57 of Rhizoctonia solani also caused severe damage to oat seedlings at high temperatures. Samuel and Garrett (8) and Sprague (11) have reported R. solani as causing some injury to oats, but the symptoms described in no case seem to be as severe as was observed in the present study. It is possible that isolates reported elsewhere were not as virulent as the one obtained by the writer. Of the 12 isolates tested, only isolate R-57 was strongly pathogenic. This indicates that isolates of R. solani differ greatly in their pathogenicity to oats. Isolate R-57 attacked a number of varieties of oats and also wheat, barley, rye, and soybeans. The wide host range and the severity of infection of this isolate on oats and rye suggest that it might at times become destructive. However, the fact that R. solani can attack only young seedlings at high temperature may limit its destructiveness.

Literature Cited

1. Bennett, F. T. 1928. On two species of Fusarium, F. culmorum (W. G. Sm.) Sacc., and F. avenaceum (Fries) Sacc., as parasites of cereals. Ann. Appl. Biol. 15: 213-244.
2. Downie, A. R. 1940. Root rot and damping-off of oats. M. S. Thesis, University of Minnesota. 39 p.
3. Hynes, H. J. 1937. Studies on Rhizoctonia root rot of wheat and oats. New South Wales Dept. Agr. Sci. Bul. 58.
4. Meehan, F., and H. C. Murphy. 1946. A new Helminthosporium blight of oats. Science 104: 413-414.
5. Melhus, I. E., J. N. Martin, C. S. Reddy, W. F. Buchholtz, and H. C. Murphy. 1940. Influence of Pythiaceous and other fungi on seedling stands of legumes and other crops. Rept. Iowa Agr. Exp. Sta. 1939-40: 140-141.
6. Meredith, C. H. 1940. A quick method of isolating certain phycomycetous fungi from soil. Phytopathology 30: 1055-1056.
7. O'Brien, D. G., and R. W. G. Dennis. 1933. Helminthosporium disease of oats. The West Scotland Agr. College Dept. Plant Husb. Res. Bul. 3.
8. Samuel, G., and S. D. Garrett. 1932. Rhizoctonia solani on cereals in South Australia. Phytopathology 22: 827-836.
9. Simmonds, P. M. 1928. Studies on cereal diseases. III. Seedling blight and foot rot of oats caused by Fusarium culmorum. Dominion of Canada Dept. Agr. Bul. 105 (n. s.).
10. Sleeth, B. 1945. Agar medium and technique for isolating Pythium

free of bacteria. Phytopathology 35: 1030-1031.
11. Sprague, R. 1937. A further note on the fungus causing a white foot rot of wheat and oats. Phytopathology 27: 798-800.
12. Welch, A. 1942. Further studies on Pythium injury of oats. Phytopathology 32: 19.

DEPARTMENT OF PLANT PATHOLOGY AND BOTANY, INSTITUTE OF AGRICULTURE, UNIVERSITY OF MINNESOTA, ST. PAUL 1, MINNESOTA

INTERFERENCE AND SYNERGY, -- THEIR POSSIBLE USE IN IDENTIFYING CERTAIN MOSAIC VIRUSES OF CEREALS AND INDICATING DEGREES OF RELATIONSHIP

H. H. McKinney[1]

ABSTRACT

A very mild strain of wheat streak-mosaic virus, when established in wheat plants, has effected complete blocking of infection by a virulent or yellowing strain of the same virus. Synergistic reactions occurred in the plants when the viruses of wheat streak mosaic, barley stripe mosaic, and brome mosaic were combined in pairs to give dual infections. In comparison with the single-virus controls, the plants with the dual infections were more stunted, headed later or failed to head, and developed more severe chlorosis. There were more curved internodes when wheat streak- mosaic virus was in the combination. The blocking or protection test appears to be of value in identifying virus strains that have been studied in cereals. Synergistic reactions in dual virus infections appear to signify that the relationship between the viruses is more distant than that between strains. The synergy test seems to be of some aid in the identification of the viruses studied.

METHODS

The tests were conducted in a shaded greenhouse from April to August. Temperatures ranged between 60° and 75° F. during the night and on overcast days, but occasionally rose above 100° F. on sunny days. The plants were grown in fertile soil in 6-inch clay pots, 6 plants to a pot. Young plants in the three-leaf stage were inoculated with the first virus, and as soon as systemic symptoms were established part of the plants were inoculated with a second virus. The remaining pots served as single-virus controls, and healthy plants of the same age were inoculated with the second virus used in the dual-infection tests. Non-inoculated controls also were included in the tests. Each dual inoculation, and each single-virus control inoculation involved 6 plants. Pots were placed 20 inches apart, center to center, in square formations.

RESULTS

Interference among Strains of the Wheat Streak-Mosaic Virus

The symptoms of the virulent streak-mosaic virus have predominated completely when wheat was inoculated with equal amounts (by weight) of leaf tissue from plants infected with the virulent or yellowing strain, and from plants infected with the very mild strain of the wheat streak-mosaic virus. Further study is necessary to determine the courses of the two strains in the plant. However, when the very mild strain (ATCC No. 85)[2] was established in Rescue, C.I. 12435, spring wheat, the very mild symptoms remained unchanged when the infected plants were inoculated with a virulent or yellowing strain (ATCC No. 29) 9 days after the first inoculation. Healthy control plants of the same age were inoculated at the same time with the virulent strain, and typical yellow streak mosaic developed in all the plants. Throughout the test, the plants receiving two inoculations expressed very weak chlorotic markings that were very difficult to discern in many of the leaves, and were indistinguishable from the markings in the leaves of the single-virus control plants that were inoculated only with the very mild strain of virus.

When inoculum taken from the plants that were reinoculated with the virulent strain was tested in healthy index plants, the resulting symptoms were of the very mild wheat streak mosaic, with no signs of the virulent strain, or of evidence of a blend of the two strains. From

---

[1]Plant Pathologist, Field Crops Research Branch, Agricultural Research Service, U. S. Department of Agriculture. Acknowledgment is due Henry J. Breen and Lester W. Greeley for assistance in conducting these studies.

[2]Culture numbers assigned in the Catalogue and Registry of the Plant Viruses, the American Type Culture Collection. Washington, D. C.

the results presented, it appears that when the very mild strain of wheat streak-mosaic virus is established in the plant, it blocks systemic infection by the virulent strain completely.

In the event that blocking of systemic infection is found to be general among the strains of the wheat streak-mosaic virus, the mildest strain and the most virulent strain can serve a useful purpose in identifying the full range of strains that differ in their characteristic degrees of virulence.

Synergistic Reactions from Dual Infections with Viruses That Differentiate above the Strain Level

Synergistic reactions resulting from dual infections with the barley stripe-mosaic virus and the brome mosaic virus have been reported (2). The present report deals with dual infections with the wheat streak-mosaic virus in combination with the barley stripe-mosaic virus and with the brome mosaic virus.

Sources of the virus strains are indicated below.

Barley stripe-mosaic virus:
- California virulent strain (BSMV-Calvir); an exceptionally virulent selection from A. Herbert Gold's culture (1); used in the blocking test illustrated in another report (2).
- Pilot avirulent strain (BSMV-Pav.); exceptionally mild, and latent in many leaves es of the susceptible wheats and barleys tested; isolated from a seedling of Pilot spring wheat that became infected through the seed; used in the blocking and synergy tests illustrated in another report (2).

Brome mosaic virus (BMV):
- Type culture, ATTC No. 66; used in the synergy test reported (2).

Wheat streak-mosaic virus:
- Virulent or yellowing strain (WSMV-vir); type culture, ATCC No. 29.
- Very mild strain (WSMV-vm), ATCC No. 85.

In the dual infections, the virus combinations are numbered as follows:

Combination 1 WSMV-vm and BSMV-Calvir
do 2 WSMV-vm and BMV
do 3 BSMV-Pav and WSMV-vir
do 4 BMV and WSMV-vir
do 5 WSMV-vir and BSMV-Calvir

Michigan Amber, C.I. 11770, a winter wheat, and Rescue spring wheat were used in two independent tests. Michigan Amber was included only in the first combinations, whereas Rescue was included in all the combinations listed above and in Table 1. The first comparisons were made on June 11 after the onset of the chronic phase of the diseases, when the Michigan Amber plants were in the tillering stage, and the healthy Rescue plants were starting to head. The observations are recorded below.

Single virus controls:	
WSMV-vm, BSMV-Pav, BMV.	Chlorotic leaf markings exceedingly weak, very few or none.
WSMV-vir.	Chlorotic leaf markings strong and numerous.
BSMV-Calvir.	Chlorotic leaf markings few in Michigan Amber, numerous and strong in Rescue.

The infected Rescue plants that expressed the exceedingly weak leaf markings were retarded about 10 percent in the length of the leaves and the stems, and the plants that expressed the strong chlorotic leaf markings were retarded about 20 percent, in comparison with the healthy controls. The infected Michigan Amber plants reacted in a similar manner, but the retardations were reflected only in the length of the leaves, since stem elongation had not started.

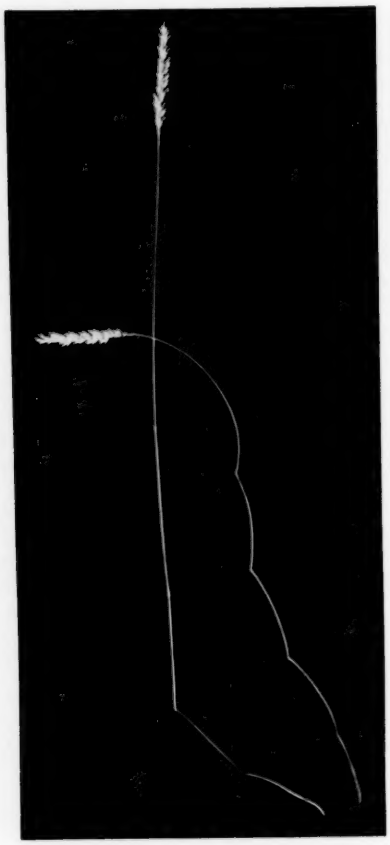


FIGURE 1. Left-lodged culm from a healthy Rescue wheat plant showing the straight upper internodes. Right--culm from a Rescue wheat plant infected with viruses of virulent wheat streak-mosaic and Pilot avirulent barley stripe-mosaic (combination 3), showing the curved upper internodes and the leaning position.

Dual infections:

Combination 3.	No striking synergistic reactions; more breaking in the chlorotic stripes than in controls, otherwise symptoms resembled those in the WSMV-vir. control.
Combinations 1, 2, 4, 5.	Strong synergistic reactions; chlorosis and stunting were accentuated in comparison with the single-virus controls.
Combinations 1, 5.	When BSMV-Calvir was in the combination, chlorosis was most extreme in the test; leaves narrow; shoots flaccid and spindly; tiller proliferation normal.
Combinations 2, 4.	When BMV was in the combination, chlorosis was less than in combinations 1 and 5, but the tillers and primary shoots were the shortest in the tests; tiller proliferation was excessive in Rescue, giving the plants a rosetted appearance; Michigan Amber plants with combination 2 were dying; in Rescue, the telescoping action of the blades and sheaths was impeded during the growth of the tillers, causing them to crumple and twist just above the plant crowns.

Table 1. Synergistic reactions induced by dual-virus infections in Rescue Wheat. Seeded April 23; first inoculation May 7.

Single-virus controls and dual-virus combinations	Chlorosis[a]	Av. length[a] of stalks	Seeding to heading
	percent	inches	days
Healthy control	0	24	65-67
WSMV-vm	6	15	74-78
Combination 1	70	3.5[b]	d
BSMV-Calvir	65	17	77-80
WSMV-vm	6	15	74-78
Combination 2	50	3.5[c]	97-105
BMV	3	19	75-78
BSMV-Pav	0.1	19	73-75
Combination 3	50	11	82-85
WSMV-vir	25	14	73-78
BMV	3	19	75-78
Combination 4	50	3.5[c]	86-117[e]
WSMV-vir	25	14	73-78
WSMV-vir	25	14	73-78
Combination 5	70	3.5[b]	f
BSMV-Calvir	65	17	77-80

[a]Observations recorded on June 21. The intensity of the chlorosis and the amount of the leaf surface involved were estimated, and the length of the stalks was measured to the topmost ligule.

[b]Stalks were flaccid and drooping, and the leaves narrow on June 21.

[c]Tillering was excessive on June 21.

[d]There was no sign of elongation of the peduncles on Aug. 6.

[e]On July 1, two small deformed heads appeared on very short deformed peduncles; extruded from the sides of the sheaths; three additional heads had appeared by Aug. 1.

[f]The original drooping shoots were dead by Aug. 6, and three of the plants were proliferating profusely; the leaves were very narrow, and mosaic markings were very strong; no signs of internode elongation.

With the completion of these observations, the Michigan Amber plants were discarded.

One week after the above observations were made the Rescue plants inoculated with the Pilot avirulent barley stripe-mosaic virus and the virulent wheat streak-mosaic virus (combination 3), expressed more severe chlorosis and more stunting than did the virulent wheat streak-mosaic control.

The plants were rated for the degrees of chlorosis, and the stalks were measured for length on June 21, as shown in Table 1. Data from the dual-infection combinations are listed between those from the two single-virus controls, the viruses which compose the combinations.

The dual infections accentuated chlorosis and stunting, and delayed heading as compared with the single-virus controls, especially in the combinations containing the brome mosaic or virulent barley stripe-mosaic viruses. The combinations containing brome mosaic virus were the only ones in which tillering was accentuated at the time of these observations, and they manifested the earliest unmistakable synergistic reactions on May 31. The combinations containing the California virulent strain of barley stripe-mosaic virus were the only ones in which the stalks were flaccid and drooping.

Table 2. Curving of the upper internodes in Rescue wheat when infected with the wheat streak-mosaic virus, especially in the dual infections.

Viruses	Culms	Inter-nodes	Curved internodes			Curved peduncles		
	No.	No.	No.	%	Radii	No.	%	Radii
Healthy control	19	95	16[c]	16.8	4-7	0	-	-
BSMV-Pav	21	105	14[c]	13.3	4-15	0	-	-
BSMV-Calvir	24	120	16[c]	13.3	5-10	0	-	-
BMV	22	110	25[c]	22.7	5-10	0	-	-
WSMV-vm	29	147	52	35.4	3-10	0	-	-
WSMV-vir	31	151	74	49.0	5-10	4	12.9	7-10
Combinations:								
1. WSMV-vm & BSMV-Calvir	7	28	28	100.0	2-7	0	-	-
2. WSMV-vm & BMV	12	40	16	40.0	5-15	6	50.0	10-15
3. BSMV-Pav & WSMV-vir	25	125	68	54.4	4-20	13	52.0	4-15
4. BMV & WSMV-vir	5	23	16	69.6	5-15	3	60.0	e
5. WSMV-vir & BSMV-Calvir	d	-	-	-	-	-	-	-

[a]Radii of the curves are in inches; measurements recorded Aug. 6.

[b]Percentage is based on total number of peduncles.

[c]Curving confined to the two lowermost elongated internodes.

[d]See footnote f, Table 1.

[e]Curves were irregular; one peduncle was in the form of a corkscrew spiral.

Curving of the upper internodes became evident in Rescue plants infected with wheat streak-mosaic virus, especially in the dual-infections. Curving of the two lowermost elongated internodes is common in a small percentage of the culms of healthy plants, but it is unusual for the upper internodes to curve appreciably, even when the culms have lodged. Curving characteristics were compared in the several inoculation tests on August 6, and the notes are recorded in Table 2. In Figure 1, a culm with curved-internodes is compared with a lodged culm. Curving was confined to the two lower internodes in all plants, with the exception of those infected with wheat streak-mosaic virus, alone or in a combination. More of the upper internodes were curved when the wheat streak-mosaic virus was in combination with one of the other viruses. It is evident that curving of the upper internodes in these tests is a characteristic sign of the wheat streak-mosaic virus. One peduncle was twisted in the form of a corkscrew.

The general appearance of the plants suggests that the wheat streak-mosaic virus influences the hormone content of the tissues. As with culms that lodge, the nodes elongated on their under sides in their response to the curving of their subtended internodes; this helped to keep the culms elevated in the general direction of the perpendicular. However, the plants had a bushy appearance as most of the culms with curved upper internodes tended to droop.

The culms of wheat plants manifesting the severe stunting and chlorosis from streak mosaic frequently tend to spread apart, giving the plants a bushy appearance. Abnormal curving of the upper internodes may account for some of this spreading in field-grown streak-mosaic plants, but it is possible that curving of the very short lower internodes may contribute to the outward bending of the culms.

Any consistent synergistic reactions characteristic of the different virus combinations should have some value in virus identification. It is noteworthy that the synergistic reactions in these tests, and in those reported earlier (2), occurred only when the dual infections involved viruses that were regarded as being distantly related, rather than closely related strains.

Inoculation tests with Golden Giant sweet corn have shown higher infectivity of the brome mosaic virus when in combination with wheat streak-mosaic virus, than when alone in the single-virus control. However, the leaf tissues from the two sources differed so greatly in physical texture, the infectivity tests may reflect little or no information on the actual difference in the quantities of virus produced.

Discussion and Conclusions

When a virus already established in a plant has the ability to bring about complete blocking of infection by another virus to which the plant is known to be susceptible, with very few exceptions, the two viruses have been differentiated at the strain level by other criteria. As a result, this blocking or protecting characteristic is generally regarded as an indication that a very close relationship between the virus isolates is highly probable.

From the results presented here and elsewhere (2) [3], it is concluded that the blocking or protection test, as used in these studies, provides additional evidence in support of the view that the viruses of very mild wheat streak mosaic and virulent or yellow wheat streak mosaic are closely related strains, and that the viruses of Pilot avirulent barley stripe mosaic and California virulent barley stripe mosaic, likewise are closely related strains.

The capacity for inducing marked synergistic reactions in the plant by two virus isolates in a dual infection, so far as known, occurs only with viruses that by other criteria differentiate at the species level or at some higher level in a systematic scheme. From the results presented here and elsewnere (2), it seems highly probable that the synergistic reactions provide further evidence that the viruses of wheat streak mosaic, barley stripe mosaic, and brome mosaic differentiate at some level or levels above that of strain, and that these relationships are therefore more distant than those between strains.

Many investigators accept the idea of virus relationship at the strain level. However, when evidence for relationship at the strain level is negative, frequently it is stated without qualification, that the viruses under consideration are unrelated, in spite of the fact that they do have certain characteristics in common, and therefore are distantly related in some categories in any systematic arrangement that can be devised. Fundamentally, systematic schemes are based on the concept of degrees of relationship between the several entities embraced--with difference and likeness tending to be complementary. The recognition and evaluation of relationships in several categories is necessary in the development of systematic virology. To attain this end, a positive approach is essential, and many apparent relationships should be accepted, even though some of these later may be found to be of little value. Without exception, this procedure with the process of revision, constitutes the basis for progress in the development of every systematic arrangement or classification.

Literature Cited

1. Gold, A. Herbert, Coit A. Suneson, Byron R. Houston, and John W. Oswald. 1954. Electron microscopy and seed and pollen transmission of rod-shaped particles associated with the false stripe virus disease of barley. Phytopath. 44: 115-117.
2. McKinney, H. H. 1956. Interference and synergistic reactions with respect to barley stripe-mosaic virus and brome mosaic virus. Plant Dis. Reptr. 40: 520-523.

FIELD CROPS RESEARCH BRANCH, AGRICULTURAL RESEARCH SERVICE,
U. S. DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND.

[3] In plants given the blocking test with strains of the barley stripe-mosaic virus, bio-assays have revealed the presence of the avirulent strain only, even in the leaves that were inoculated with the challenge virus.

VIRUSES IN THE HYBRID LILY L. T. A. HAVEMEYER

Frank P. McWhorter [1]

Especially desirable among the lilies developed recently for home-garden use are hybrids between Lilium henryi Baker and various trumpet lilies. One of the first of these interspecies crosses was a hybrid between L. henryi and L. myriophyllum var. superbum (Baker) E. H. Wilson, made in New Jersey in 1933. This cross, introduced under the name L. T. A. Havemeyer, soon became an expensive item in the trade. The lily L. T. A. Havemeyer established a vogue for this kind of lily that combined the disease resistance of L. henryi with the less resistant but more attractive trumpet lilies such as L. sargentiae Wilson, L. leucanthum var. chloraster (Baker) Wilson, and other trumpet forms. The original Havemeyer hybrid remained very high-priced and was never generally established in American commercial plantings or home gardens.

Shortly after the variety Havemeyer was introduced, a bulb was planted in an isolated area at Newberg, Oregon, and grown into large clumps that now bloom at an average height of approximately 8 feet. The foliage of these lilies has for years had a mild diffused mottle unlike the conspicuous square mottle characteristic of L. henryi. The usual virus of L. henryi is distinct from the other viruses of lily [2] and is very different from the mottle virus of L. myriophyllum var. superbum, the other parent of L. T. A. Havemeyer. The L. henryi virus induces coarse yellow patches in the foliage and reddish blotches on the flowers of the pink test-tulip, Clara Butt.

Beginning in 1955 the virus complex responsible for the mottling of the foliage of the Havemeyer hybrid was analyzed by inoculations to Clara Butt tulips. The inoculations were made to determine whether the hybrid was infected with the henryi virus or the more usual mottle viruses widely distributed among lily species. Nine tulips were inoculated May 25, 1955, at a time when the foliage of the tulips was too mature for most effective transfer. The transfer noted, however, during April 1956 was 4/9, a high incidence for late-season inoculations. Three of the four infected tulips bore whitened flowers and strongly striped leaves; one had a dark-red flower and normal leaves. This proves that the L. T. A. Havemeyer hybrid is susceptible to and is carrying the lily color-removing virus and the lily color-adding virus instead of the virus characteristic of L. henryi.

Since this isolated Havemeyer planting dates back to a very early purchase, it is probable that all bulbs of these lilies contain this mottle virus complex which makes them undesirable for planting near trumpet lilies that can be injured by these viruses [2]. The hybrids, however, grow with amazing vigor in spite of this virus infection and are, therefore, suitable for planting alone or in locations where susceptible lilies or tulips would not be exposed, or in locations where only lilies that "can live with virus" are grown.

OREGON AGRICULTURAL EXPERIMENT STATION AND UNITED STATES DEPARTMENT OF AGRICULTURE, AGRICULTURAL RESEARCH SERVICE

---

[1] Pathologist, Oregon Agricultural Experiment Station, Corvallis, and Agent, United States Department of Agriculture.

[2] McWhorter, Frank P., and Philip Brierley. 1955. The Principal Virus Disease of Lilies. The Lily Yearbook 1955, pp. 10-27.

DISTRIBUTION OF CABBAGE YELLOWS IN LOUISIANA IN 1955

Eugene C. Tims and Ralph T. Brown[1]

Cabbage yellows (Fusarium oxysporum f. conglutinans (Wr.) Snyder & Hansen) was first reported in Louisiana near Breaux Bridge in 1948 (4). Evidence has been accumulated since that time to indicate that the disease had probably existed in the State at an earlier date. Yellows had little opportunity to develop in the State because most of the cabbage was grown in late fall, winter and early spring when temperature conditions were unfavorable for the disease to develop. However, some cabbage was planted early enough to encounter conditions favorable for yellows development in September and October. The first report of the disease came in October and subsequent occurrences were noted in September and October.

In October 1953, several diseased cabbage plants were sent to Dr. W. J. Martin, at Baton Rouge from Plaquemines Parish (south of New Orleans). He isolated cultures of Fusarium from these plants and turned them over to the senior author who conducted pathogenicity tests with the All Year cabbage variety. All the cultures caused typical yellows symptoms in the cabbage seedlings within ten days. The diseased plants had been taken from a cabbage variety test plot at the Plaquemines Parish Experiment Station where yellows was well established. Susceptible varieties such as Winnigstadt and Imported Glory were severely affected.

In October 1955, diseased cabbage plants were received from St. Martin Parish by Dr. A. G. Plakidas who identified the disease as cabbage yellows.

The farm where the plants came from was visited a few days later. Several acres of Copenhagen Market and Glory of Enkheizen were found to be infected with yellows. From 10 to 20 percent of the plants were dead or severely affected. The owner of this farm said he had seen the disease on his place for the past four or five years in late spring or early fall cabbage.

Recently Ralph Brown summarized the yellows situation in Plaquemines Parish by writing[2] that "Yellows infested soils are widespread in the cabbage growing area of this parish." He is of the opinion that the fields have been infested for some years. But it has only been in comparatively recent times that cabbage was planted early in the fall and kept in the fields late in the spring when conditions were favorable for yellows development.

A number of yellows resistant cabbage varieties have been grown in an infected plot at the Plaquemines Parish Experiment Station, in tests to determine the best varieties for local use. In the early tests most of the resistant varieties held up very well. In the 1955 test the Marion Market, Resistant Globe, Resistant Glory, and Medium Copenhagen Resistant varieties showed a high degree of resistance. But in all the tests the Resistant Detroit showed moderate susceptibility. Eddins (2) stated that the amount of yellows had been greatly reduced in Florida by the introduction of such resistant cabbage varieties as Marion Market, Resistant Medium Copenhagen, Resistant Glory and Resistant Detroit. Walker and Smith (5) found that there may be a gradual stunting and death of the plants of resistant varieties at 28° C. and above. Excessively high temperatures in the field might have accounted for the disease development in the Resistant Detroit variety in the 1955 test in Louisiana.

Where did yellows come to Louisiana from and when did it first appear? The writers have been unable to determine the answer to either question definitely. Weber (6) in 1936 reported that yellows was probably introduced to the Bartow section of Florida from Racine, Wisconsin. Later Eddins and Burrell (1) stated that the disease was probably introduced to the Hastings area in Florida in diseased plants from Georgia and Virginia. These writers saw diseased cabbage plants being carried into one field in the Hastings area.

Ishaque (4) in the first report of the disease in Louisiana stated that no explanation could be given at that time as to how and when the fungus was introduced. One grower in St. Martin Parish has imported cabbage plants from Florida and Mississippi. This farmer obtained some plants from Palatka, Florida, which is only a few miles from Hastings, where the disease is rather widespread (1). The same grower also obtained plants from Mississippi. Yellows has been known to occur in that State as early as 1934 (3).

Yellows is known to occur in only two parishes in Louisiana at the present time: Plaquemines Parish, southwest of New Orleans and St. Martin Parish, southwest of Baton Rouge. The disease probably occurs in other sections of South Louisiana where it has not been reported.

---

[1] Respectively Plant Pathologist, Louisiana Agricultural Experiment Station, Baton Rouge, and Superintendent, Plaquemines Parish Experiment Station, Diamond, Louisiana.

[2] Personal Correspondence.

Literature Cited

1. Eddins, A. H. and S. M. Burrell. 1949. Occurrence of cabbage yellows in the Hastings Section, Florida. Plant Dis. Reptr. 33: 249-251.
2. Eddins, A. H. 1952. Distribution of cabbage yellows in Florida in 1952. Plant Dis. Reptr. 36: 337-338.
3. Black, L. M. 1934. Uniformity in pathogenicity and cultural behavior among strains of the cabbage yellows organism. Jour. Agric. Res. 48: 401-409.
4. Ishaque, M. 1949. Occurrence of cabbage yellows in Louisiana. Plant Dis. Reptr. 33: 233.
5. Walker, J. C. and R. Smith. 1930. Effect of environmental factors upon resistance to cabbage yellows. Jour. Agric. Res. 41: 1-15.
6. Weber, G. F. 1937. Two new fungous diseases of vegetables in Florida. Plant Dis. Reptr. 15: 282.

LOUISIANA AGRICULTURAL EXPERIMENT STATION, BATON ROUGE, LOUISIANA AND PLAQUEMINES PARISH EXPERIMENT STATION, DIAMOND, LOUISIANA.

SEED TRANSMISSION OF RHIZOCTONIA SOLANI IN PHASEOLUS VULGARIS AND P. LUNATUS

Charles M. Leach and Merle Pierpoint

In January of 1956 a sample of beans in a roll towel germination test at the Oregon State Cooperative Seed Testing Laboratory was observed to be deseased. Rhizoctonia solani Kuehn was isolated from the large, reddish-brown, sunken lesions that were abundantly present on the hypocotyls (Fig. 1).

FIGURE 1. Bean seedlings with lesions caused by Rhizoctonia solani.

Following this first observation of Rhizoctonia-infected beans, 211 samples have been routinely tested for germination; of these 22 percent developed lesions characteristic of Rhizoctonia solani. Infection from seed-borne Rhizoctonia has been noted in the following varieties of Phaseolus vulgaris and P. lunatus: Asgrow Stringless, Black Valentine, Logan Improved, White Seeded Tendergreen, Top Crop, Antwerp, McCaslan Pole, Kentucky Wonder 191, King Green, Woodruff's Green, Dixie White Butterpea, Genuine White Runner, Wade Bush, Woodruff Hyscore, Giant Stringless Green Pod, Seminole, Henderson Bush, Cangreen U. S. 1, Jackson Wonder, Woodruff's Prolific Bush, Henderson Bush Green Cotyledon, Fordhook Woodco, Fordhook 242, Willow Leaf Lima, Giant Butter Speckle, King of the Garden, and Burpee's Improved Bush. All seed samples so far examined have been from Idaho and California.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, OREGON AGRICULTURAL EXPERIMENT STATION; AND OREGON STATE COOPERATIVE SEED TESTING LABORATORY, CORVALLIS.

HOST RANGE STUDIES ON TOBACCO ANTHRACNOSE CAUSED BY A SPECIES OF COLLETOTRICHUM[1]

Omar D. Morgan, Jr. [2]

Introduction

Tobacco anthracnose is known to occur in different areas of the world. Averna-Sacca (1) first reported it from Brazil in 1922 and named the causal fungus Colletotrichum nicotianae Averna-Sacca. Boning (2, 3) found anthracnose in Germany in 1929 and named the causal organism C. tabacum Boning. Dickson (6) reported a Colletotrichum on tobacco from Australia in 1929. Boning (4) isolated a Gloeosporium from tobacco with typical anthracnose lesions in 1933. Nakamura (14) in 1934 reported anthracnose of tobacco from Japan. Johnson and Valleau (7, 8) in 1935 showed that the source of an outbreak of anthracnose in plant beds at the Kentucky Experiment Station was from alfalfa. They determined that the disease was caused by C. destructivum O'Gara. Matsumatra (9) in 1946 reported a tobacco anthracnose from Formosa and said it was caused by C. tabacum.

Since it was first reported in the United States, anthracnose has been found in Maryland and Virginia by Walker (17, 18), Pennsylvania by Burke (5), and Nusbaum (15) reported it from North Carolina. Walder (19) found that a Colletotrichum and a Gloeosporium were associated with the disease. McGrew (10, 12) gives an extensive review of tobacco anthracnose.

Anthracnose was generally distributed over the tobacco areas in Maryland by 1942 (17). Since then it has been found every year in varying degrees of severity. The disease caused considerable damage to tobacco plant beds in the spring of 1945 (19), in 1950, and again in 1952; occurrence in the latter 2 years was observed by the author. Anthracnose caused more damage in 1950 than either blue mold or wild fire and it was nearly as severe in 1952. Both of these years followed relatively mild winters. The disease was found in the field in both years though it was not serious.

Sources of the disease have been reported by Tsumagari (16) as debris carried with the seed and infected stalks left in the seed bed from the previous year's crop. The author has observed the disease in Maryland as being associated with infected stalks left in plant beds, but anthracnose has also been observed in Maryland in new plant beds and where there was no previous history of the disease. The lack of any known source indicated that the disease may have come from hosts other than tobacco.

Anthracnose has generally not been a problem where growers have dusted or sprayed plant beds with ferbam, zineb, or manzate. Greenhouse tests by the author indicate one application of these chemicals will control anthracnose for as long as one month providing the material is not washed off.

The host range of the Colletotrichum sp. causing anthracnose of tobacco in Maryland has been studied by Walker (19), McGrew (10, 11, 12), and Morgan (13). Walker (19) experimentally infected pokeweek, tomato, irish potato, geranium, and begonia. McGrew (10, 11) produced infection in 42 species of Nicotianae and 14 named varieties of commercial tobacco. McGrew (12) showed that some of the Nicotiana species were moderate to highly resistant. Morgan (13) reported on species of crop and weed plants susceptible to infection by the fungus causing tobacco anthracnose. Host range studies were undertaken in 1951 and 1952 in the greenhouse and in 1952 in the field to determine sources of the Colletotrichum sp. causing tobacco anthracnose. Many of the plants used in the tests were found growing in or around tobacco plant beds.

Materials and Methods

Seedlings of 48 species representing 16 families as follows: 14 from Graminae, 8 from Leguminosae, 6 from Solanaceae, 6 from Compositae; 2 each from Cruciferae and Chenopodiaceae; 1 each from Phytolaccaceae, Rubiaceae, Umbelliferae, Caryophyllaceae, Bignoniaceae, Cucurbitaceae, Labiatae, Geraniaceae, Bigoniaceae, and Convolvulaceae, were used in the inoculation tests (see Table 1 for species).

Most plants used in the tests were grown from seed or seed-pieces in 3-inch pots, either alone or in small numbers in each pot. Cuttings and weed plant seedlings growing in and around

[1] Scientific Article No. A583, Contribution No. 2746 of the University of Maryland Agricultural Experiment Station, Department of Botany.

[2] Assistant Professor of Plant Pathology.

Table 1. The host range of a Colletotrichum sp. causing tobacco anthracnose as determined in greenhouse and field tests and as naturally infected in plant beds.

Latin Binomial	Common Name	Variety	Greenhouse test*	Field test*	Naturally infected in field*
Graminae		Not determined for grasses			
Agrostis alba L.[a]	Red top		+ Gr 2**	+	0
Bromus inermis Leyes[a]	Brome grass		+ Gr 2	+	0
Lolium perenne L.[a]	Perennial rye grass		+ Gr 2	+	0
Sorghum vulgare pers.[a]	Common sudan grass		+ Gr 2	+	0
Hordeum vulgare L.[a]	Barley		-	0	0
Agrostis palustris Huds.[a]	Creeping bent grass		-	0	0
Triticum sativum Lam.[a]	Wheat		-	0	0
Arrhenatherium elatius (L.) Presl.[a]	Tall meadow oat		-	0	0
Phalaris canariensis L.[a]	Canary grass		-	0	0
Poa pratensis L.[a]	Kentucky blue grass		-	0	0
Festuca elatior L.[a]	Tall fescue		-	0	0
Dactylis glomerata L.[a]	Orchard grass		-	0	0
Cynodon dactylon (L.) pers.[a]	Wire grass		-	0	0
Unknown grass[a]			0	0	+
Leguminosae					
Medicago sativa L.[a]	Alfalfa	Buffalo	+ Gr 1 & 3	+	0
Pisum sativum L.[a]	Peas		+ Gr 1	0	0
Soja max (L.) Pifer[a]	Soybean	Lincoln	+ Gr 1	0	0
Trifolium pratense L.[a]	Red clover	Kenland	+ Gr 1 & 3	+	0
Trifolium repens L.[a]	Ladino clover	Ladino	+ Gr 1 & 3	0	0
Phaseolus vulgaris L.[a]	String bean	Stringless green pod (bush)	-	0	0
Vigna sinensis Savi.[a]	Cow pea		-	0	0
Crotalaria spectabilis Roth.[a]	Crotalaria		-	0	0
Solanaceae					
Nicotiana tabacum L.[a]	Tobacco	Catterton	+ Gr 1	+	+
Lycopersicum esculentum Mill.[a]	Tomato	Marglobe	+ Gr 1	+	+
Datura stramonium L.[a]	Jimson weed		+ Gr 1	+	+
Capsicum annuum L.[a]	Pepper	California Wonder	+ Gr 1	+	+
Solanum tuberosum L.[b]	Irish potato	Katahdin	+ Gr 1	0	0
Solanum carolinense L.[c]	Horse nettle		-	0	0

Table 1. (Continued).

Compositae					
Lactuca sativa L.[a]	Leaf lettuce	Slobolt	+ Gr 3	+	+
Lactuca sativa L.[a]	Head lettuce	Iceberg	+ Gr 3	+	+
Lactuca canadensis L.[c]	Wild lettuce		+ Gr 3	0	0
Helianthus annuus L.[a]	Common sunflower		+ Gr 1	0	0
Taraxacum officinale Weber[c]	Common dandelion		-	0	0
Bellis perennis L.	Daisy	English daisy	-	0	0
Chenopodiaceae					
Beta vulgaris L.[d]	Sugar beet	Improved sugar	+ Gr 1	+	0
Chenopodium album L.[c]	Lambs quarter		-	0	0
Cruciferae					
Brassica Rapa L.[a]	Turnip	Purple top white globe	-	0	0
Brassica oleroacea var. Capatato L.[a]	Cabbage		-	0	0
Bigoniaceae					
Begonia L. sp.[e]	Begonia		+ Gr 1	0	0
Bignoniaceae					
Campsis radicans Seem.[c]	Trumpet creeper		-	0	0
Caryophyllaceae					
Stellaria media (L.) Cyrill.[c]	Common chickweed		-	0	0
Cucurbitaceae					
Cucumis sativus L.[a]	Cucumber	Straight eight	+ Gr 1	0	0
Geraniaceae					
Pelargonium L'Her. sp.[a]	Geranium		+ Gr 1	0	0
Labiatae					
Coleus Loun. sp.[e]	Coleus		+ Gr 1	0	0
Phytolaccaceae					
Phytolacca americana L.[c]	Pokeweed		-	0	0

Table 1. (Concluded).

Rubiaceae					
Cephalanthus occidentalis L.[c]	Button bush		-	0	0
Umbelliferae					
Daucus carota L. var. Sativa DC[a]	Carrot	Danvers half long	-	0	0
Convolvulaceae					
Ipomoea Batatas Lam.[e]	Sweet potato	Maryland Golden	-	0	0

[a] Plants grown from seed
[b] Plants grown from seed pieces
[c] Plants transplanted from field
[d] Plants grown from roots
[e] Plants grown from cuttings

* The (+) sign indicates positive infection, (-) sign indicates no infection, and (0) plants not tested or found infected in field.
** Gr. 1, 2, & 3 refer to groups as given in text.

tobacco beds were pulled and transplanted into 3-inch pots (Table 1). The soil and the pots were steam sterilized. Roots of whole plants brought from the field were washed before transplanting to sterile soil.

A culture of the Colletotrichum sp. was isolated from tobacco and used throughout the tests, which covered a 2-year period. Numerous transfers did not decrease it pathogenicity.

Four potted plants of each species or four pots with a number of seedlings were inoculated in each test. The same number of uninoculated checks were used. The series of inoculations was repeated twice in the greenhouse over a 2-year period.

The plants were inoculated with a spore suspension of the fungus. The spores were sprayed on the plants with a de Vilbis atomizer attached to a small electric motor-driven pressure pump.

After inoculation the plants were put into a moist chamber and kept wet for 6 days at temperatures of 72° to 75° F. After 6 days they were removed from the chamber. Isolations were made from all plants showing the disease. Tobacco was inoculated directly from infected plants, or the organism was isolated on potato dextrose agar, then inoculated to tobacco to determine the pathogenicity of the isolates. Microscopic examinations were made of the lesions on infected plants and dead tissue of uninfected plants for setae and spores.

Field tests were made in tobacco plant bed sites at the University of Maryland Tobacco Research Farm. Seed of 12 species and varieties that had shown symptoms of anthracnose in the greenhouse inoculation tests was sowed in 1-yard-square plots. The 12 species and varieties were inoculated on 4-week old seedlings with a spore suspension of tobacco anthracnose fungus. Observations were made 10 days after inoculation. The field test was run once during the spring of 1952.

Results

The results of inoculation studies are summarized in Table 1. Reaction of susceptible plants to the fungus was not always the same on the different species. The infected plants were grouped into three categories (13). Group 1 was the leaf spot, vein, petiole, and stem lesion group. Most of the broadleaf plants were included in group 1. Group 2 was the dry rot reaction group and included grasses only. Group 3 was a wilt reaction group and included lettuce, alfalfa, clovers, and overlapped in group 1 on tomato, jimson weed, and pepper.

The group 1 infected plants showed the typical anthracnose leaf spot, stem, petiole, and vein lesions. Some variation occurred in size, color, and position of lesions on the plant. Those susceptible plants that had leaf spots and lesions on stem petiole and vein were tobacco (Fig. 1-G), tomato (Fig. 1-A and D), jimson weed (Fig. 1-B and D), and pepper (Fig. 1-C and D), also mature clovers and alfalfa, peas, and sugar beet (Fig. 1-H). Lesions were not found on petioles or stems of sunflower, soybean, coleus, geranium, begonia, or cucumber. Pokeweed did not show infection on potted plants. Walker (19) infected pokeweed by the detached leaf method; this was not used in the above tests.

Leaf spots varied in size from 1 to 2 millimeters on cucumber and sunflower to 8 millimeters on tobacco, jimson weed, beets, peas, tomato, coleus, and geranium. Leaf spots on other susceptible plants varied between the above measurements.

The leaf spots in most species generally started as small water-soaked areas. Later collapse of the tissues in the spot occurred, followed by a slightly raised ridge limiting the size of the spot. Once fully formed, the spots did not increase in size. The collapsed tissue turned either brown or grey; both types could be found on the same leaf.

The Colletotrichum sp. used caused a frogeye type of leaf spot on sugar beet (Fig. 1-H). The spots had grey centers surrounded by a reddish ring which in turn was surrounded by a light green area. Leaf spots on tomato were somewhat irregular. When a number of the spots coalesced, the tomato leaflets would curl (Fig. 1-A). Water-soaking was not conspicuous on leaves of soybean, cucumber, and sunflower; possibly due to the small size of the spot. Spots on these plants were usually brown.

Stem, petiole, and leaf vein showed small sunken elongate or oval lesions. The tissue breaks down and turns a dark green to black. Size of the lesions varied on the same plant and between species from short pencil-like marks to lesions up to 1/2 x 1/8 inch. Lesions on stem and petiole and vein increased in size as the plant grew.

Terminal infection occurred on some plants resulting in death of the growing tip. Flowers became infected if they were present; this was particularly true of wild lettuce (Fig. 1-K). Tomato, pepper, and jimson weed were susceptible to terminal infection (Fig. 1-A, B and C).

The second or dry rot group included only the grasses. The symptom was caused by the fungus attacking the base of the leaf blade, leaf sheath, and stem (Fig. 1-I). Death was caused by girdling of the stem. Acervuli with setae occurred in abundance on leaf blade and sheath, much

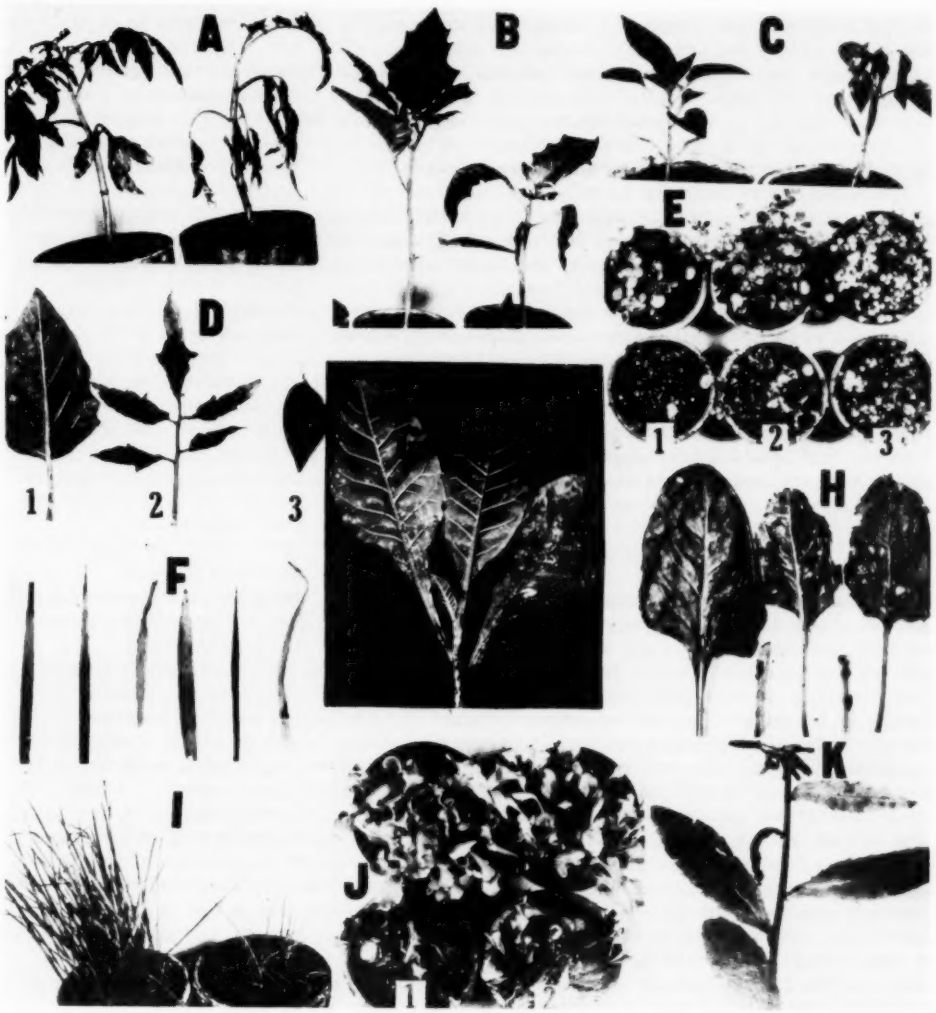


FIGURE 1. Hosts of tobacco anthracnose. A. Tomato healthy, right, and diseased left. B. Jimson weed healthy, right, and diseased, left. C. Pepper healthy, right and diseased, left. D. Leaves of A, B, and C showing lesions on petiole and leaf tissue. E. Clovers and alfalfa healthy, top, and diseased, below. E-1. Ladino clover, E-2. Red clover, and E-3 Alfalfa. F. Shows lesions on leaves of sudan grass. G. Tobacco. H. Lesions on sugar beet leaf and petiole. I. Sudan grass healthy, right, diseased, left. J. Leaf. J-1. Head, J-2. Lettuce healthy, top, diseased, below. K. Wild lettuce, diseased flowers and leaves.

the same as those found in grasses infected with Colletotrichum graminicola (Ces.) Wils. The leaf blade would dry and turn brown. Leaf spots were scarce on the leaf blade (Fig. 1-F). The spots were small and turned brown or purple.

The two lettuce varieties, alfalfa, and red and ladino clover formed the third group and were the only plants that showed a definite wilting (Fig. 1-E and J.). The fungus attacked the crown and lower portion of leaf or petiole of lettuce varieties resulting in a wilt. The fungus attacks the crown and stem of alfalfa and clover seedlings, girdling the stem or petiole and causing a wilt and death of the seedling. Mature alfalfa and clovers had leaf spots, stem and petiole lesions. The older plants were not killed.

Setae and spores appeared only on dead tissue of the lesions but not on all lesions. Setae and spores could be found developing on dead tissue of plants not infected, indicating that the fungus is saprophytic on many dead plants. Setae and spores were scarce on lesions of cucumber and abundant on dead chickweed tissue.

Isolations of the fungus made from the infected plant species and varieties and inoculated on tobacco leaves produced typical anthracnose spots and lesions. Isolates from dead plant tissue of non-susceptible plants also produced typical anthracnose spots and lesions.

On the 12 species and varieties grown in the tobacco seed bed (Table 1) and inoculated with the Colletotrichum causing tobacco anthracnose, all became infected. Infection in the bed was not as severe as in greenhouse tests due to hot dry weather conditions. Heavy infection was observed on leaf and head lettuce, sugar beets, red clover, alfalfa, pepper, and jimson weed. Tomato and brome and sudan grass were moderately infected while soybeans had a few scattered spots on the leaves.

Discussion

The evidence presented in this report indicates that the Colletotrichum sp. causing tobacco anthracnose attacks a wide variety of weeds and crop plants. Many of these hosts are found in and around tobacco beds.

It has been the practice of some growers to start tomatoes and peppers in the plant beds and to grow lettuce in or near the beds. Such a practice makes these plants excellent sources of anthracnose on tobacco. Field observations indicate that whenever, a severe outbreak of the disease occurs where such plants as tomatoes, peppers, lettuce, some grasses, and jimson weed are present, they may become infected. Although a number of weed hosts infected with the fungus have been associated with tobacco, it is not known which was infected first.

The type of infection caused by the Colletotrichum sp on certain of the hosts, as dry rot on grasses and wilt on lettuce, is possibly the reason these plants were overlooked in the past as hosts. Other hosts infected experimentally, as soybeans and clover, have been used as cover crops; however, neither of these have been found infected in beds or in fields nearby.

Information obtained from the tests made has been used in the field to find the hosts of tobacco anthracnose. Such plants as lettuce, tomato, pepper, jimson weed, and an unknown grass have been found naturally infected in plant beds.

The fact that the fungus causing anthracnose of tobacco grew on the dead tissue of uninfected plants indicates that a good source of the fungus would be on old plant debris.

Literature Cited

1. Averna-Sacca, R. 1922. Fungi causing disease of tobacco in Brazil. Bul. Agr. (São Paulo) 23: 201-268.
2. Boning, K. 1929. Die Brennfleckenkrankheit des Tabaks. Prakt. Blatt. Pflanzenb. u. Pflanzensch. 7: 36-40.
3. Boning, K. 1932. Die Bekämpfung der Brennfleckenkrankheit des Tabaks (Colletotrichum tabacum) durch Beizung des Samens und vorbeugende Behändlung der Pflanzen mit chemischen Mitteln. Prakt. Blatt. Pflanzenb. u. Pflanzensch. 10: 89-106.
4. Boning, K. 1933. Uber eine zweite Brennfleckenkrankheit des Tabaks, hervorgerufen durch einem Pilz aus Gattung Gloeosporium Desmaz. et Mont. Prakt. Blatt. Pflanzenb. u. Pflanzensch. 10: 253-255.
5. Burke, O. D. 1942. Disease information for the middle Atlantic States (compiled by R. S. Kirby). Tobacco anthracnose - Pennsylvania. Plant Dis. Reptr. Suppl. 140: 36.

6. Dickson, B. T. 1929. Report of the work of the Division of Economic Botany for the year 1928-1929. Pamplet Council Sci. and Indust. Res. Australia 14: 29 pp.
7. Johnson, E. M., and W. D. Valleau. 1935. Tobacco dis-diseases in Kentucky. Plant Dis. Reptr. 19: 144.
8. Johnson, E. M., and W. D. Valleau. 1936. Tobacco diseases. Kentucky Agr. Exp. Sta. Bul. 362.
9. Matsumatra, T. 1946. Tobacco diseases in Formosa. Mem. Fac. Agr. Taiwan Univ. 1: 1-26.
10. McGrew, J. R. 1951. Susceptibility of several species and varieties of Nicotiana to tobacco anthracnose. Masters Thesis, Univ. of Maryland. 51 pp.
11. McGrew, J. R. 1952. A preliminary screening of the genus Nicotiana for resistance to tobacco anthracnose. (Abs.) Phytopathology 42: 343.
12. McGrew, J. R. 1954. Comparison of some Nicotiana species and hybrids for resistance to tobacco anthracnose. Doctors Thesis. Univ. of Maryland. 31 pp.
13. Morgan, O. D. 1955. Reaction of various hosts to the fungus causing anthracnose of tobacco. (Abs.) Phytopathology 45: 465.
14. Nakamura, H. 1934. Tobacco anthracnose. (In Japanese). Hatano Expt. Sta. Bul. 33: 54-71.
15. Nusbaum, C. J. 1951. The occurrence of tobacco anthracnose in North Carolina. Plant Dis. Reptr. 35: 276.
16. Tsumagari, Hikatoshi. 1952. Studies on anthracnose, a leaf spot disease of tobacco. Kagoshima Tobacco Experiment St. Bul. Kagoshima Tobacco Exp. Sta. 7: 1-100.
17. Walker. E. A. 1942. Tobacco seed bed diseases in Maryland. Tobacco anthracnose. Plant Dis Reptr. 26: 243
18. Walker, E. A. 1944. Anthracnose of tobacco in Virginia. Plant Dis. Reptr. 28: 838.
19. Walker, E. A., and Elizabeth Wise McInvoy. 1945. Tobacco anthracnose, a plant bed and field disease. Phytopathology 35: 598-601.

DEPARTMENT OF BOTANY, UNIVERSITY OF MARYLAND AGRICULTURAL EXPERIMENT STATION

TRUNK, BRANCH, AND STEM CANKER OF COFFEE TREES

Eddie Echandi and R. H. Segall[1]

SUMMARY

The canker disease of coffee incited by Ceratostomella fimbriata has been found for the first time in Costa Rica. The disease is characterized by chlorosis, defoliation, and eventual death of coffee stems, branches, and the tree itself. Inoculations with the fungus resulted in production of typical symptoms. Cross inoculation tests with the rubber and coffee strains of the fungus on these two crops failed to give symptoms. Observations made seem to indicate that the disease has been present on coffee trees in the main coffee growing regions for a long period.

A disease of coffee trees caused by Ceratostomella fimbriata, characterized by the formation of cankers on the trunks, branches, and stems, has been found for the first time in Costa Rica. The symptoms of the disease and the causal organism are similar to the ones reported by Pontis (2) from Colombia and Venezuela and by Szkolnik (3) from Guatemala.

The disease has been found on coffee trees from 10 to 50 years old but more commonly on older trees. The earliest symptom is a chlorosis of the leaves on stems near the canker. The chlorotic leaves drop and the stem dies. Branches both above and below the original canker may be killed (Fig. 1), and the whole tree may eventually die a few months or more than a year after the first symptoms appear. Diseased coffee trees often have no visible canker.

FIGURE 1. Coffee tree with canker at base and dying branches above canker.

FIGURE 2. Bark removed from trunk with black discoloration of canker.

However, if the bark is removed, a red-brown to black discoloration is found in the wood. This discoloration does not extend deeply into the wood. The cankers vary in size and shape, and can appear in almost any woody part of the tree, but are most commonly found in proximity to pruning wounds (Fig. 2). Most of the diseased trees observed have been heavily pruned and most of the pruning has been done about one foot above the soil surface. On 5- year-old trees cankers could be detected without removing the bark because the stems or branches were

[1] Professor of Plant Pathology, University of Costa Rica, and Plant Pathologist, United States Operations Mission to Costa Rica, International Cooperation Administration.

FIGURE 3. Long necked perithecium of Ceratostomella fimbriata X about 75.

FIGURE 4. Extrusion of hat shaped ascospores from neck of perithecium X about 337.5

completely girdled. Pruning of dead branches of heavily diseased trees sometimes stimulates the production of new shoots, but these are weak and carry only a few abnormally small berries.

Observations made in some of the coffee farms of the Central Plateau indicated that the disease has been present in the country for a long period, but since it appears most commonly on old plants, it has been overlooked or confused with other diseases.

Isolations made from cankers yielded a fungus identical to Ceratostomella fimbriata. Perithecia of Ceratostomella were formed on potato dextrose agar at room temperature 4 days after seeding (Fig. 3). These perithecia exuded hat shaped ascospores (Fig. 4). Asci were not observed. Hyaline continuous conidia were abundent. Brown thick-walled conidia were present in 20-day-old cultures.

Two-year-old shoots of coffee trees were inoculated with 3-day-old cultures of C. fimbriata in potato dextrose agar by making a vertical cut into the shoot about 5 inches from the base and inserting the mycelium. The wounds were then covered with plastic tape. Ten shoots were inoculated in this manner, and potato dextrose agar alone was inserted in control shoots. Necrosis began to appear in the border of the wounds 3 days after inoculation, extending rapidly both up and down from the point of inoculation. The cankers varied in size and shape. In several of the cankers perithecia appeared, extruding spores 11 days after inoculation. In all cases the fungus could be reisolated from the cankers. The leaves on some of the stems close to the point of inoculation wilted and became chlorotic after 14 days, and fell after 30 days. In addition, it was experimentally demonstrated that the fungus entered through wounds usually made during pruning.

Ceratostomella fimbriata causes a rot of tapping panel of rubber trees (1). The rubber isolate when inoculated on coffee grown at various altitudes failed to produce symptoms. Likewise the inoculations made with the coffee isolate on rubber trees were unsuccessful. A comparison made between the coffee and rubber isolates on potato dextrose agar showed morphological differences between the two isolates.

When the disease was first found, a survey was made of the main coffee growing areas of Costa Rica to determine the extent of spread of the disease. Diseased trees were found in most of the areas. The greatest disease incidence was found on coffee grown at the highest altitudes. Between 3500 and 5000 feet up to 60 percent of the trees were infected. Between 2500 and 3500 feet some farms had up to 30 percent of the coffee trees diseased, and below 2500 feet the disease incidence was less than 5 percent. Representative mean annual temperatures at the various elevations are: Cartago, 4732 feet, 66° F.; San Jose, 3828 feet, 69°; Turrialba, 1980 feet, 72°. Inoculations made at altitudes of 300, 2000 and 3500 feet above sea level were successful. Farms in which the humidity was higher than average usually due to excessive shade

or poor soil drainage had a higher incidence of disease.

Literature Cited

1. Martin, W. J. 1949. Moldy rot of tapping panels of Hevea rubber trees. U. S. Dept. Agr. Circ. 798. 23 p.
2. Pontis, R. 1951. A canker disease of coffee trees in Colombia and Venezuela. Phytopathology 41: 178-183.
3. Szkolnik, M. 1951. Coffee trunk and stem canker in Guatemala. Plant Dis. Reptr. 35: 500-501.

UNIVERSITY OF COSTA RICA AND INTERNATIONAL COOPERATION ADMINISTRATION, UNITED STATES OPERATIONS MISSION TO COSTA RICA, AGRICULTURE AND NATURAL RESOURCES BRANCH

LONGEVITY OF SPORES OF THE OAK WILT FUNGUS ON OVERWINTERED NITIDULID BEETLES[1]

William J. Stambaugh and Charles L. Fergus[2]

ABSTRACT

The longevity of conidia and ascospores of the oak wilt fungus, Endoconidiophora fagacearum Bretz, in or on dead or dormant Nitidulid beetles overwintered in the forest duff under natural conditions was studied. Spore viability was determined, using the spermatization technique. The insects used were collected from fresh mycelial mats formed in nature. Some of the mats bore perithecia. In addition, some insects were artificially contaminated with spores. Conidia in or on insects survived a maximum of 94 to 121 days, whereas ascospores survived 151 or more days. It is concluded that insects coming in contact with ascospores on mycelial mats formed in the fall are carrying viable spores at the end of their winter hibernation. These spores could probably serve as inoculum during the spring period when oaks are resuming cambial activity -- the supposed period of maximum susceptibility and infection.

INTRODUCTION

Longevity of the spores of Endoconidiophora fagacearum Bretz is an important factor to be considered in determining the mode and limits of overland transmission of the oak wilt disease. An abundance of inoculum is generally available in nature during the spring and fall months when mycelial mats are produced on the boles of trees. Experiments using radioactive substances (8) have offered definite proof that insects (chiefly Nitidulidae) which frequent these mats are also attracted to fresh wounds on healthy oaks.

Results of natural wound transmission studies in Pennsylvania (2), Iowa (10), and Wisconsin (6), and insect inoculations of wounded oaks (3, 5, 6, 9, 13), substantiate an earlier report (1) that maximum susceptibility to and infection by the oak wilt fungus occurs during the spring period of mat formation which usually coincides with the resumption of cambial activity in oaks. The true significance of fall mat formation is difficult to evaluate, although successful inoculations during the fall months have indicated that oaks are susceptible to infection during this period (12).

It has been demonstrated that Nitidulid beetles carry viable spores in or on their bodies after contact with mat inoculum (11, 14) and that conidia remain viable in the fecal material after passage through the intestinal tract of these beetles (4). Although controlled experiments have shown that optimum survival of both conidia and ascospores occurs under cool, dry conditions (7), longevity of spores in nature in or on the bodies of suspected vectors has not been determined.

During the winter of 1955-56, Nitidulid beetles from fall mat collections were stored in the duff layer of the forest floor to determine spore longevity under conditions as natural as possible.

PROCEDURE

During the period October 19 to November 14, 1955, insects were collected from mature mycelial and perithecial mats, and separated by species into lots of 10 to 25. Each lot was placed in a flat, plastic screen cage. The cages were placed in the duff under the bole of a

---

[1] Contribution No. 216 of the Department of Botany and Plant Pathology. Authorized for publication Sept. 11, 1956, as paper No. 2091 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Instructor and Associate Professor of Botany and Plant Pathology. Appreciation is expressed to Dr. T. L. Guyton, Director, Bureau of Plant Industry, Pennsylvania Department of Agriculture for suggestions which initiated this study. The aid of Dr. H. E. Thompson, Entomologist, Pennsylvania Department of Agriculture, in collecting and identifying the insects, and the assistance of Mr. Ralph Sanderson, Pennsylvania Department of Agriculture, in the field work is gratefully acknowledged.

Table 1. Percent insects carrying viable spores of Endoconidiphora fagacearum after varying intervals of overwintering in forest duff layer.

Insect	Source of Insect[a]	Percent Insects Testing Positive At Start[b]	After Intervals of Time[c]	Time interval (Days)
Colopterus niger	M	100	67	30
			0	183
Colopterus truncatus	M	35	17	30
			33	92
			0	107
			0	152
Carpophilus sayi	M	100	50	30
Eupurea spp.	M	90	100	65
Rhizophagidae spp.	M	6	20	37
			0	61
Mixed species	M		57	58
			0	74
Colopterus truncatus	P	43	40	36
			0	86
Rhizophagidae spp.	P		0	86

[a] M indicates insects were collected from a mycelial mat with conidia only.
P signifies perithecia had been produced.

[b] A minimum of 10 insects were tested in each case.

[c] 10 to 25 insects were tested at each interval.

felled tree. The storage plot was located on a well drained slope having a southern aspect. Cages were removed for testing at approximately 30-day intervals throughout the winter and early spring (December 2, 1955 to May 16, 1956). Duff, soil surface, and air temperatures were recorded at periodic intervals during the storage period.

To supplement these collections and standardize more equally the spore concentration in determining longevity, dead Nitidulid beetles from stump trap collections were artificially contaminated with conidia and ascospores. Conidia were applied externally by rubbing the beetles thoroughly over the surface of a sporulating conidial culture. Ascospores were applied by placing three ascosporic masses on the ventral body surface of each insect. Thirteen insects bearing conidia were placed in each of five plastic screen cages. The insects bearing ascospores were treated in a like manner. All of these cages were placed in the duff storage plot on December 16, 1955. One cage for each spore type was removed for testing at approximately 30-day intervals thereafter until May 16, 1956.

Immediately after removal the cages were returned to the laboratory, where spore viability in or on the bodies of the insects was determined by the spermatization technique

Table 2. Percent[a] artificially contaminated insects carrying viable spores of Endoconidiophora fagacearum after varying intervals of winter storage in the duff layer.

	Storage Period in Days					
	0	31	62	94	121	151
Insects With:						
Conidia	100	92	62	15	0	0
Ascospores	100	100	85	31	15	8

[a] Percentage based on tests of 13 insects.

described in a previous publication (11). Spore longevity in this study is recorded as the percentage of insects yielding viable spores of the oak wilt fungus after various storage intervals. As a check on the initial percentage of insects carrying viable spores, some insects from each mat collection date were tested immediately (Table 1). Insects that were artificially exposed to conidia and ascospores were also tested before winter storage (recorded as 0 days storage in Table 2).

RESULTS AND DISCUSSION

The maximum longevity of conidia in the two experiments reported here was 92 (Table 1) and 94 (Table 2) days, a remarkably close agreement. Previous in vitro studies (7) indicated that conidia could survive 173 days at the most optimum temperature and relative humidity studied. However, these conditions did not fluctuate as they probably do in the forest.

Ascospores survived 151 days of storage in the duff (Table 2) when placed artificially on insects. Since this was the last attempted viability determination and 8 percent of the insects still carried viable spores, it is probable that the ascospores would remain viable for even longer periods. It therefore appears probable that ascospores formed in the fall on mycelial mats would survive the winter in or on dormant insects in the forest duff.

The temperature of the duff remained fairly constant on the study plot, being 0° C throughout January and February and 1° to 3° C through March. The air temperature above the duff was taken each noon and was found to range from 1° to 11° C during these months. The moisture and humidity relations of the duff are possibly very important in their effects on spore longevity, particularly since they would fluctuate diurnally and even hourly. This is indicated by the results of previous in vitro studies (7) which illustrated the lessened longevity of spores stored at high humidities as compared with those stored at low humidities at the same temperature.

Observations were also made on the survival of the insects collected from naturally formed mats. The insects varied greatly in their ability to survive storage in the plastic mesh cages in the duff, even among individuals of the same species. Spermatization tests were made with both dead and living insects, and positive results were obtained from both.

Inconsistent results were obtained from the insects collected from naturally occurring mats. Furthermore, there were several unexpected results which are difficult to explain (Table 1). For example, conidia apparently remained viable longer than ascospores. In addition, the numbers of Colopterus truncatus carrying viable spores that were collected from perithecial mats were not significantly greater than those collected from mycelial mats without perithecia and ascospores. Actually, the data for insects collected from mycelial mats with perithecia, thus presumably carrying ascospores, is unfortunately not extensive enough to make valid comparisons.

When insects were artificially contaminated with spores, results were more consistent and the fungus lived longer (Table 2). One possible explanation for this is that these insects received the maximum number of spores in the best possible physiological state for maximum

longevity. The age, viability, physiological state, and numbers of spores on insects collected from mats were unknown.

Study of Table 1 also reveals the interesting result that a large number of insects collected from fresh sporulating mats did not give positive spermatization tests. For example, only 35 percent of Colopterus truncatus specimens and 6 percent of the Rhizophagidae were carrying viable spores when first collected.

In conclusion, conidial inoculum picked up by insects from mycelial mats produced in the fall fails to survive the winter on hibernating insects, whereas ascospore inoculum remains viable.

Literature Cited

1. Craighead, F. C., and C. L. Morris. 1952. Possible importance of insects in transmission of oak wilt. Forests and Waters 4(6): 126-129.
2. _____, C. L. Morris, and J. C. Nelson. 1953. A preliminary note on the susceptibility of wounded oaks to natural infection by the oak wilt fungus. Plant Dis. Reptr. 37: 483-484.
3. Himelick, E. B., E. A. Curl, and B. M. Zuckerman. 1954. Tests on insect transmission of oak wilt in Illinois. Plant Dis. Reptr. 38: 588-590.
4. Jewell, F. F. 1954. Viability of the conidia of Endoconidiophora fagacearum Bretz, in the fecal material of certain Nitidulidae. Plant Dis. Reptr. 38: 53-54.
5. _____. 1956. Insect transmission of oak wilt. Phytopathology 46: 244-257.
6. McMullen, L. G., C. R. Drake, R. D. Shenefelt and J. E. Kuntz. 1955. Long distance transmission of oak wilt. in Wisconsin. Plant Dis. Reptr. 39: 51-53.
7. Merek, E. L., and C. L. Fergus. 1954. The effect of temperature and relative humidity on the longevity of the oak wilt fungus. Phytopathology 44: 61-64.
8. Morris, C. L., H. E. Thompson, B. L. Hadley Jr., and J. M. Davis. 1955. Use of radioactive tracer for investigation of the activity pattern of suspected insect vectors of the oak wilt fungus. Plant Dis. Reptr. 39: 61-63.
9. Norris, D. M., Jr. 1953. Insect transmission of oak wilt in Iowa. Plant Dis. Reptr. 37: 417-418.
10. _____. 1955. Natural spread of Endoconidiophora fagacearum Bretz to wounded red oaks in Iowa. Plant Dis. Reptr. 39: 249-253.
11. Stambaugh, W. J., C. L. Fergus, F. C. Craighead, and H. E. Thompson. 1955. Viable spores of Endoconidiophora fagacearum from bark and wood-boring beetles. Plant Dis. Reptr. 39: 867-871.
12. Stambaugh, W. J., and J. C. Nelson. 1956. Observations concerning autumn infection of oak by Endoconidiophora fagacearum Bretz. Plant Dis. Reptr. 40: 750-751.
13. Thompson, H. E., B. L. Hadley, Jr., and A. R. Jeffery. 1955. Transmission of Endoconidiophora fagacearum by spore-infested Nitidulids on wounded healthy oaks in Pennsylvania. Plant Dis. Reptr. 39: 58-60.
14. Yount, W. L., A. R. Jeffery, and H. E. Thompson. 1955. Spores of Endoconidiophora fagacearum on the external surfaces of the body of Nitidulids. Plant Dis. Reptr. 39: 54-57.

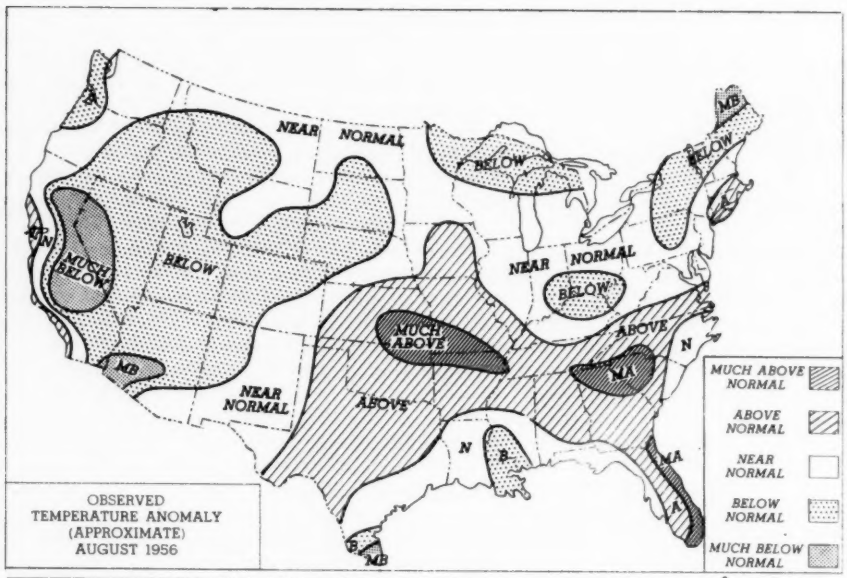
DEPARTMENT OF BOTANY AND PLANT PATHOLOGY, PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PENNSYLVANIA

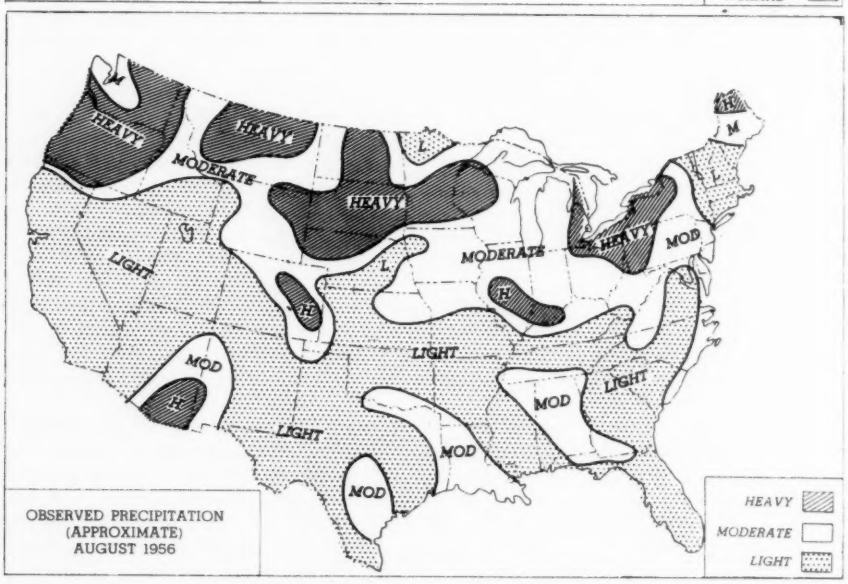
ANNOUNCEMENT:

"RESULTS OF 1955 FUNGICIDE TESTS"

"Results of 1955 Fungicide Tests" reprinted from a series of articles appearing in AGRICULTURAL CHEMICALS, April through June, May be purchased in bound and covered form for $1.00 per copy by sending orders with remittance to Dr. A. G. Newhall, Department of Plant Pathology, College of Agriculture, Cornell University, Ithaca, New York. The publication of these results is under the sponsorship of the American Phytopathological Society. It is a continuation of the publication of results formerly provided through a Supplement of the PLANT DISEASE REPORTER, Plant Disease Epidemics and Identification Section, U. S. Department of Agriculture.

The Temporary Advisory Committee on Collecting and Disseminating Data on New Fungicide Tests of the American Phytopathological Society arranged for the recent publication of data and the continuation of a program for annual publications of Fungicide Test Results in the future. Dr. A. G. Newhall, Department of Plant Pathology, Cornell University, Ithaca, New York, will be in charge of this project during the current year.





The terms used in the accompanying maps, which define the ranges of temperature and precipitation, are numerical class limits. These are based on a statistical analysis of past records through which is determined the normal frequency of occurrence of temperatures and precipitation at various times of the year for different locations. For temperature the classes above, below, and near normal are so defined that they each normally occur one-fourth of the time; much above and much below normal, one-eighth of the time. Precipitation is depicted in terms of light, moderate, and heavy, each class normally occurring one-third of the time and thereby having equal probability of occurrence. These maps graphically represent only the general trends and give the country's weather picture at a glance. For quantitative studies, where monthly mean temperatures and actual precipitation records are needed for a given time and place, other publications of the Weather Bureau should be consulted. P. R. M.

MANUSCRIPTS FOR THE PLANT DISEASE REPORTER

(See also PDR 35: 121-122; 36: 214, 494)

(1) GENERAL: The Reporter page measures 9 inches long with the heading or 8 3/4 inches for the text part, by 6 inches wide. The copy is typed on a larger page, 11 1/4 inches of text or 12 inches overall in length by 8 inches in width, and reduced 25 percent in the photographic process of reproduction. Illustrations or tables larger in either dimension will take a correspondingly greater reduction. Only one size of type is available for text, footnotes, or tables.

(2) MANUSCRIPTS should be the original ribbon copy, not carbons, clearly typed and double-spaced throughout, including tables, footnotes, and bibliographies. (Note -- only one copy is needed.) Footnotes should be typed at the bottom of the page.

(3) ABSTRACTS are requested for all except very short articles. (PDR 38: 316).

(4) CAUSES OF DISEASES should be named. For bacteria, fungi, nematodes, etc., give the Latin name of the organism; for viruses either or both the accepted common name of the virus or a Latin name if you prefer it and there is one; for non-parasitic diseases state the causal factor if it is known. If the cause of a disease has not been determined say so. (PDR 36: 494).

(5) LITERATURE REFERENCES should be given in alphabetical order and numbered for citation in the text. We follow the AIBS suggestion of placing the year of publication after the author's name. Please check your titles carefully since we cannot do it for you. Be sure that text citations and bibliography agree; that foreign-language references are correct; that number or month is cited for periodicals that are not paged consecutively throughout the volume.

(6) NAMES OF FUNGICIDES should be given according to the suggestions that will be published in Phytopathology before long, and later repeated in the Reporter. In the meantime see PDR 35: 122.

(7) ILLUSTRATIONS should be sent to us unmounted. To prevent mistakes, write figure numbers on the back, and mark the top of each print when necessary. A sketch can show a preferred arrangement but please keep in mind page size, shape, and standard reduction (see above under General), and remember that figure titles and legends are part of the page. Lettering should be clear and large enough to be legible after reducing. Drawings, maps, and graphs can be photographs or originals, but should be finished and ready for reproduction, not just sketches.

(8) TABLES should be carefully thought out with particular attention to the Reporter's limitations in reproduction. Make titles and headings definite and self-explanatory. Designate footnotes in tables with superscript lower-case letters. Be sure that text discussion agrees with the data in the table. Do not abbreviate names of crop varieties.

(9) REPRINTS cannot be supplied since there is no way in which we can be reimbursed. However,

(10) The MULTILITH PLATES from which reprints can be made will be sent if requested at the time the article is submitted. The press size of these plates used for the Reporter is designated as small -- maximum image 9 1/2 by 13 inches, maximum paper size 9 3/4 by 14 inches -- for Model 1250. Most of the Experiment Stations have this type of multilith machine.

(11) We are the official correspondents in the United States of the PLANT PROTECTION BULLETIN published monthly by the United Nations Food and Agriculture Organization. We would appreciate it if plant pathologists would send us suitable material from time to time for inclusion in our monthly summary reports. Please mark it clearly so that we will know it is intended for the Bulletin. (See PDR 36: 492; 37: 637) -- P. R. M.